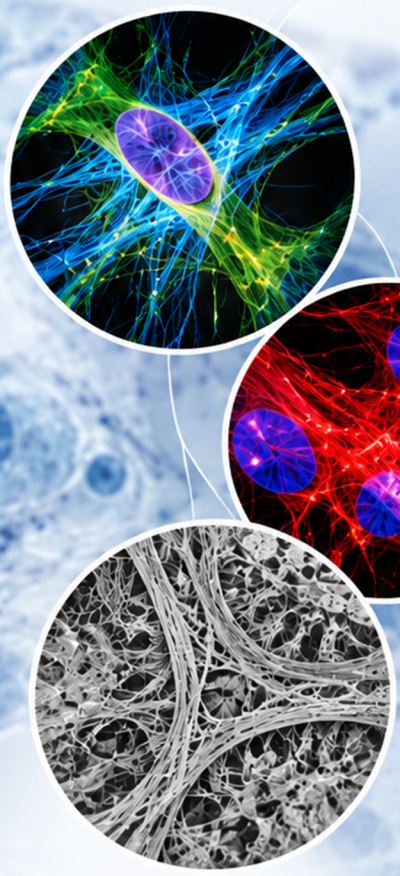
# HISTOLOGICAL ASPECTS OF CYTOSKELETAL DISORDERS:

## FROM MOLECULAR MECHANISMS TO HUMAN DISEASE



Editors
Dr. Seda KESKİN
Dr. Elif KAYHAN

DUVAR

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## From Molecular Mechanisms to Human Disease

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**Preface**

The cytoskeleton is far more than the structural scaffold of the cell. Comprising actin microfilaments, microtubules, and intermediate filaments, along with their associated motor proteins and nuclear partners, this dynamic network governs cell shape, polarity, division, migration, intracellular transport, and mechanotransduction. Defects in its components therefore underlie a remarkably diverse spectrum of human disease, from congenital malformations and ciliopathies to neurodegeneration, cancer and infertility.

Over the past two decades, advances in molecular genetics, high-resolution imaging and functional cell biology have transformed our understanding of how cytoskeletal dysfunction gives rise to tissue pathology. Histology remains essential, as it reveals how molecular defects are reflected in cellular and tissue architecture. It is central to diagnosis, to the interpretation of experimental models and to the teaching of the medical sciences.

***Histological Aspects of Cytoskeletal Disorders: From Molecular Mechanisms to Human Disease*** comprises nine chapters arranged in a logical progression. The opening chapters address disorders of actin microfilaments and intermediate filaments, as well as of the dynein, kinesin and myosin motor proteins. The following chapters examine primary ciliopathies and laminopathies, highlighting the roles of the cytoskeleton at the cilium and the nuclear envelope. The final chapters consider cytoskeletal dysfunction in developmental and congenital disorders, neurodegenerative diseases, cancer progression and metastasis, and infertility.

Throughout the book, we evaluate molecular mechanisms, histopathological changes, and clinical implications together. This integrative approach underscores that diseases with distinct clinical presentations may share common mechanisms rooted in disrupted cellular organization, structural integrity and mechanical regulation. By linking molecular abnormalities to their morphological and functional consequences, the book aims to provide a coherent scientific framework for understanding the role of cytoskeletal dysfunction in human pathology.

We sincerely thank all contributing authors for their expertise, dedication, and scholarly rigour. We hope that this book will serve as a valuable reference for histologists, pathologists, clinicians, researchers and graduate students, and that it will inspire further research bridging basic cell biology, tissue pathology and clinical medicine.

**Dr. Seda Keskin**
**Dr. Elif Kayhan**
**Editors**

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# Chapter 1

## Actin Cytoskeleton Disorders: Molecular Mechanisms and Clinical Manifestations

**Hilal Nakkas**[1*,2]

**Abstract**

The actin cytoskeleton is a highly conserved and dynamically regulated system in architecture, migration, intracellular organization, mechanotransduction, and tissue-specific functions. Genetic disruption of actin and associated partners gives rise to a diverse group of disorders referred as actinopathies. This chapter provides an integrated overview of the molecular, cellular, histological, and clinical features of selected actinopathies by summarizing the structural organization and isoform diversity of human actins, the major actin-binding and regulatory proteins, and the developmental processes. The disease mechanisms and structural consequences of pathogenic variants in actin associated genes were examined. Current approaches to molecular diagnosis, experimental disease modeling, and therapeutic management are also discussed. The chapter concludes by emphasizing the importance of genotype–phenotype correlations and mutation-specific therapeutic strategies in managing actinopathies. Collectively, these disorders illustrate how disruption of a common cytoskeletal machinery can produce highly tissue-specific diseases and provide a framework for understanding the actin cytoskeleton as a mechanistically coherent target for precision medicine.

**Keywords:** Actin cytoskeleton; Actinopathies; Actin isoforms; Actin-binding proteins; Cytoskeletal disorders

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## 1. Introduction

Actin is one of the most abundant and highly conserved proteins in eukaryotic cells. Humans express six highly related actin isoforms: β-cytoplasmic actin gene (ACTB) and γ-cytoplasmic actin gene (ACTG1), which encode the ubiquitously expressed cytoplasmic β- and γ-actins, and skeletal muscle α-actin gene (ACTA1), smooth muscle α-actin gene (ACTA2), cardiac α-actin gene (ACTC1), and smooth muscle γ-actin gene (ACTG2), which encode predominantly skeletal-muscle, vascular-smooth-muscle, cardiac-muscle, and visceral-smooth-muscle actins, respectively. β- and γ-cytoplasmic actin differ at only four of 375 amino-acid positions, yet their expression, localization, and interactions with actin-binding proteins are not fully interchangeable (Clayton et al., 2024; Di Donato et al., 2026). This chapter uses the term *actinopathy* to describe monogenic disorders caused by pathogenic variants in an actin gene or in a principal actin-regulatory partner. In this chapter, particular emphasis is placed on sarcomeric, cytoplasmic, and podocyte actinopathies whose clinical phenotypes have been well-characterized and underlying mechanisms thoroughly elucidated.

Because each actin isoform and its associated regulatory machinery is deployed in a specific tissue context, mutations that disrupt fundamentally the same molecular process, actin polymerization, filament cross-linking, or filament–motor interaction, produce strikingly different clinical syndromes depending on which gene and which cell type is affected. Dominant missense mutations in the cytoplasmic actins ACTB and ACTG1 cause Baraitser-Winter syndrome, a disorder of neuronal migration and craniofacial development; mutations in the sarcomeric actin ACTA1 cause nemaline myopathy, a severe congenital disorder of skeletal muscle; mutations in the cardiac actin ACTC1 cause hypertrophic and dilated cardiomyopathy; mutations in the actin-nucleation regulator Wiskott–Aldrich Syndrome Protein (WASP) cause Wiskott-Aldrich syndrome, a combined immunodeficiency and platelet disorder; mutations in ACTG1 affecting cochlear hair cells cause progressive autosomal dominant hearing loss (DFNA20/26); and mutations in the actin cross-linking protein α-actinin-4 (ACTN4) cause a hereditary form of focal segmental glomerulosclerosis (FSGS) affecting the kidney podocyte (Despond & Dawson, 2018; Kaplan et al., 2000; Labasse et al., 2022; Morín et al., 2009; Notarangelo & Ochs, 2003; Rivière et al., 2012). Considered together, these disorders illustrate how a single, ancient cytoskeletal system can be repurposed across radically

different tissues, and how its disruption produces a correspondingly diverse set of human diseases.

This chapter examines actin cytoskeleton disorders from several complementary angles. It begins with the normal histology of the actin cytoskeleton and the embryological processes that depend on it, then examines the molecular, histological, and cellular structure of the actin isoforms and regulatory proteins involved and the major signaling pathways that govern them under healthy conditions. It then examines how these systems fail in each disorder and the resulting histopathological and structural changes, followed by the clinical manifestations and diagnostic biomarkers these changes produce. The chapter closes with a review of the experimental models used to study these processes, the therapeutic strategies developed to date, a discussion of future perspectives, and a synthesis of the evidence.

## 2. Normal Histology and Embryological Development

### 2.1 Actin Structure and Isoform Diversity

Actin exists in a dynamic equilibrium between monomeric, ATP-bound G-actin and filamentous F-actin, a right-handed double helix of subunits with structural polarity: a fast-growing (barbed) end and a slower-growing (pointed) end. All six human actin isoforms share this basic architecture, but ACTA1, ACTA2, ACTC1, and ACTG2 are expressed predominantly in skeletal, smooth vascular, cardiac, and visceral smooth muscle respectively, where they form the thin filament of the contractile sarcomere, while ACTB and ACTG1 are expressed ubiquitously and support the branched and bundled actin networks of the cell cortex, growth cone, adherens junctions, and stereocilia (Clayton et al., 2024).

### 2.2 Actin-Binding and Regulatory Proteins

Actin filament assembly, geometry, and turnover are controlled by a large set of accessory proteins. The Arp2/3 complex nucleates branched actin networks when activated by nucleation-promoting factors such as the WASP family, which themselves are held in an autoinhibited state until activated by the Rho-family GTPase (Ras Homolog Family of Small Guanosine Triphosphatase) Cdc42 (Yarar et al., 1999). Formins nucleate long, unbranched filaments; cofilin severs and depolymerizes aged filaments to recycle actin monomers; and cross-linking proteins such as α-actinin bundle parallel filaments into mechanically resilient arrays, a function that is especially critical in the podocyte foot process, where ACTN4 organizes the cortical actin network that resists the mechanical stretch of glomerular filtration (Feng et al., 2018).

### 2.3 Embryological Development Dependent on Actin Dynamics

Each tissue affected by the actinopathies discussed in this chapter depends on actin dynamics at a specific, identifiable stage of embryonic development. In the developing cerebral cortex, ACTB and ACTG1 are required for the proliferation of ventricular zone neural progenitors; patient-derived cerebral organoids carrying pathogenic ACTB or ACTG1 variants are reduced in size and show a thinner ventricular zone due to reduced progenitor abundance, directly linking cytoplasmic actin function to the generation of cortical neurons (Niehaus et al., 2026).

In skeletal muscle, ACTA1 is incorporated into the thin filament during myofibrillogenesis and, beyond its contractile role, contributes to the structural integrity of the nuclear periphery, interacting with the nuclear lamina and Linker of Nucleoskeleton and Cytoskeleton (LINC) complex components such as lamin A/C and nesprin-1/2 during myonuclear organization (Labasse et al., 2022). In the heart, ACTC1 is incorporated into the sarcomeric thin filament from early cardiogenesis onward and, because it is also expressed in the developing atrial septum, its disruption can produce congenital structural defects in addition to later contractile dysfunction (Frank et al., 2019). In the hematopoietic system, WASP-dependent actin remodeling is required for the normal development of platelets, T cells, and other leukocytes, and for the formation of the immune synapse and podosomes needed for cell migration and antigen presentation (Notarangelo & Ochs, 2003). In the developing cochlea, γ-actin is dispensable for initial hair cell development but becomes essential for maintaining the actin core of the stereocilia once these structures have formed, explaining the characteristically post-lingual onset of ACTG1-related hearing loss (Morín et al., 2009). In the developing kidney, ACTN4 organizes the actin cytoskeleton of the podocyte foot process as the glomerular filtration barrier matures, a structure that must remain both mechanically resilient and dynamically responsive to the periodic mechanical stretch of glomerular blood flow for the remainder of life (Feng et al., 2018).

## 3. Molecular, Histological and Cellular Structure and Related Major Signaling Pathways

Each actinopathy discussed in this chapter arises from disruption of a distinct, tissue-specific actin-dependent structural and signaling system. Understanding the normal organization of these systems is the necessary basis for understanding how each subsequently fails.

### 3.1 β/γ-Cytoplasmic Actin and Rho-GTPase–WASP–Arp2/3 Signaling

In non-muscle cells, β- and γ-cytoplasmic actin polymerize into branched cortical and growth-cone networks under the control of the Rho-family GTPases Cdc42 and Rac Family Small GTPase 1 (Rac1), which relieve the autoinhibited conformation of WASP-family nucleation-promoting factors and thereby license Arp2/3-mediated actin nucleation (Yarar et al., 1999). This regulatory network contributes to neural-progenitor behavior and neuronal migration during cortical development. In Baraitser-Winter syndrome, pathogenic ACTB and ACTG1 missense variants disrupt actin organization and cell behavior; the relative contribution of individual Rho-GTPase, WASP-family, and Arp2/3 components to each variant-specific disease mechanism remains under investigation.

### 3.2 Sarcomeric α-Actin and the Actin–Myosin–Troponin–Tropomyosin Signaling Complex

In striated muscle, sarcomeric α-actins (ACTA1 in skeletal muscle, ACTC1 in the heart) polymerize into the thin filament and form cyclic cross-bridges with the myosin thick filament, a process regulated by the calcium-sensitive troponin–tropomyosin complex bound along the thin filament. Under physiological conditions, contraction is tuned by the calcium sensitivity of this actin–troponin interaction and by post-translational modifications such as troponin I phosphorylation, which normally modulates the force–calcium relationship in response to β-adrenergic signaling (Hassoun et al., 2022).

### 3.3 WASP Structure and Immune Actin-Nucleation Signaling

WASP is held in an autoinhibited conformation in which its C-terminal VCA (verprolin homology, central, acidic) domain is folded back against its GTPase-binding domain. Binding of activated Cdc42 disrupts this autoinhibited state, releasing the VCA domain to engage the Arp2/3 complex and actin monomers and thereby nucleate new, branched actin filaments at sites of T-cell receptor engagement, platelet activation, and podosome formation (Notarangelo & Ochs, 2003; Yarar et al., 1999).

### 3.4 Stereociliary γ-Actin and Hair Cell Cytoskeletal Maintenance

Within cochlear hair cells, γ-actin forms the core of the stereocilia, the cuticular plate, and the adherens junctions that anchor neighboring hair cells, contributing to stereociliary shape, stiffness, and the mechanotransductive properties required for hearing. Unlike its role in most other tissues, γ-actin's function here is not primarily developmental: it is dispensable for the initial

formation of hair cell stereocilia but essential for their ongoing maintenance throughout life (Morín et al., 2009).

### 3.5 α-Actinin-4 Structure and Podocyte Actin Cross-Linking Signaling

α-Actinin-4 is an actin-bundling protein that cross-links parallel F-actin filaments through paired calponin-homology actin-binding domains, conferring mechanical resilience to the podocyte's cortical actin network. This cross-linking activity is itself regulated: epidermal growth factor (EGF) signaling can drive phosphorylation of α-actinin-4 at tyrosine residues 4 and 31, which reduces its actin-binding affinity and permits the dynamic remodeling that the mature podocyte requires to accommodate ongoing mechanical stretch (Shao et al., 2019).

## 4. Disease Mechanisms and Histopathological/Structural Changes

When the tissue-specific structural and signaling systems described above are disrupted by mutation, each actinopathy produces a characteristic cascade of cytoskeletal dysfunction and a corresponding, often diagnostic, set of histopathological or ultrastructural changes.

### 4.1 Baraitser-Winter Syndrome: ACTB/ACTG1 Dominant-Negative Mutations and Cortical Malformation

Baraitser-Winter syndrome is caused by heterozygous, almost always de novo, missense mutations in ACTB or ACTG1, including recurrent hotspot substitutions such as ACTB p.Arg196His and ACTG1 p.Ser155Phe, with the phenotypes associated with variants such as ACTB p.Thr120Ile and ACTG1 p.Thr203Met (Rivière et al., 2012). These variants are best described as altered-function missense variants; a dominant-negative contribution has been proposed for some alleles, but the precise mechanism is variant-dependent and should not be reduced to a single Rho-GTPase--WASP--Arp2/3 pathway defect. Patient-derived cerebral organoids show reduced ventricular-zone progenitor abundance, a thinner ventricular zone, and reduced organoid size, providing a plausible cellular link to the neurodevelopmental phenotype (Niehaus et al., 2026).

Cortical brain malformations occur in approximately 60–70% of affected individuals and are especially frequent in ACTG1-related disease. Pachygyria, often frontal or perisylvian, is the most common abnormality; lissencephaly and neuronal heterotopia are less common. Microcephaly may occur, but it should be described as a clinical growth phenotype rather than as a cortical malformation (Di Donato et al., 2016; Yates et al., 2017).

Distinct from these dominant missense mechanisms, loss-of-function (haploinsufficiency) variants in ACTB — for example, nonsense or frameshift mutations — produce a separate, generally milder pleiotropic developmental disorder with its own characteristic combination of facial, skeletal, and neurodevelopmental features, illustrating that the same gene can generate mechanistically and clinically distinct actinopathies depending on whether the mutation alters or abolishes protein function (Cuvertino et al., 2017).

### 4.2 ACTA1-Related Nemaline Myopathy: Actin Aggregation and Sarcomeric Disruption

Dominant and recessive ACTA1 mutations account for more than half of severe nemaline myopathy cases and disrupt normal assembly of skeletal α-actin into the sarcomeric thin filament. Rather than being efficiently incorporated into functional sarcomeres, mutant actin frequently misfolds and aggregates, producing the histopathological hallmark of the disease: nemaline rods, electron-dense, rod-shaped inclusions derived from Z-disc material that accumulate within the sarcoplasm. Muscle biopsies from severely affected patients additionally show intranuclear rods, cytoplasmic bodies, and an enlarged perinuclear space associated with mislocalization of the nuclear lamina proteins lamin A/C, nesprin-1, and nesprin-2, indicating that mutant ACTA1 disrupts nuclear as well as sarcomeric architecture (Labasse et al., 2022). Additional structural anomalies include focal, core-like areas of sarcomeric disorganization, congenital fiber type disproportion, and, less commonly, zebra bodies. Some patients with a longer clinical course show increased ACTC1 expression in skeletal muscle, suggesting partial compensatory substitution for the defective skeletal isoform (Clayton et al., 2024; Labasse et al., 2022).

### 4.3 ACTC1-Related Cardiomyopathy: Thin Filament Dysfunction

Pathogenic ACTC1 variants are a rare cause of hypertrophic cardiomyopathy and can also cause dilated cardiomyopathy, atrial-septal defects, and other cardiac phenotypes. Their effects on the thin filament vary by variant: some increase calcium sensitivity, while others decrease it or alter the normal response to troponin-I phosphorylation (Despond & Dawson, 2018; Hassoun et al., 2022).

Electron microscopy of mutant actin filaments shows grossly normal filament formation but mutation-specific differences in length, straightness, and the site of incorporation into the nascent sarcomere. Certain ACTC1 mutations produce an additional structural phenotype distinct from adult-onset contractile dysfunction: because ACTC1 is also expressed in the developing atrial septum, specific variants cause atrial-septal defects that present alongside, or years before, late-onset dilated cardiomyopathy (Frank et al., 2019; Zarate et al., 2025).

### 4.4 Wiskott-Aldrich Syndrome: Defective Actin Polymerization in Hematopoietic Cells

Wiskott-Aldrich syndrome results from loss-of-function mutations in the X-linked WAS gene, which abolish or severely reduce WASP-mediated, Arp2/3-dependent actin nucleation in hematopoietic cells. The structural consequence is a broad failure of actin-dependent cellular processes across multiple hematopoietic lineages: platelets are produced in reduced numbers and are abnormally small in size; T cells fail to reorganise their actin cytoskeleton normally during immune synapse formation, impairing antigen-receptor signaling, proliferation, and cytokine secretion; and myeloid cells show defective podosome formation and migration (Notarangelo & Ochs, 2003; Yarar et al., 1999). Because this molecular defect affects multiple hematopoietic lineages, its clinical and pathological manifestations are systemic rather than confined to a single tissue. The characteristic hematological findings include microthrombocytopenia, whereas histopathological changes may occur secondarily in association with chronic infections, eczema, and autoimmune inflammation.

### 4.5 ACTG1-Related Hearing Loss: Progressive Stereociliary Degeneration

Autosomal dominant ACTG1 missense mutations, mapping to the DFNA20/26 locus on chromosome 17q25.3, impair γ-actin polymerization within cochlear hair cell stereocilia. Because γ-actin is required for stereociliary maintenance rather than initial formation, the structural consequence is not a developmental malformation but a progressive, age-dependent deterioration of the hair cell cytoskeleton: functional and cellular studies of ACTG1 mutants demonstrate impaired actin filament assembly and disorganization of the stereociliary actin core over time, correlating with the clinical progression of hearing loss (Morín et al., 2009). Sequence analysis across reported families has identified amino acid position 118 as a particular mutational hot spot, with multiple independent substitutions at this residue producing a similar structural and clinical phenotype, consistent with a specific, localized disruption of actin filament packing rather than a generalized loss of protein stability (Wang et al., 2018).

### 4.6 ACTN4-Related FSGS: Podocyte Cytoskeletal "Freezing" and Foot Process Effacement

Autosomal dominant ACTN4 mutations affecting the actin-binding domain — including p.Lys255Glu, p.Thr259Ile, and p.Ser262Pro — paradoxically increase, rather than decrease, the protein's affinity for F-actin. The structural consequence is a pathologically "frozen" podocyte cytoskeleton: rather than permitting the dynamic

actin remodeling needed to accommodate the periodic mechanical stretch of glomerular filtration, mutant α-actinin-4 locks the cortical actin network into a rigid configuration, and podocytes expressing these mutants show extensive, irrecoverable loss of F-actin architecture and are markedly more prone to detachment from the glomerular basement membrane under mechanical load (Feng et al., 2018). This cytoskeletal failure disrupts the nephrin-supported slit diaphragm complex, producing the histopathological hallmark of FSGS: segmental sclerosis of the glomerular tuft accompanied by effacement (flattening and simplification) of the podocyte foot processes on electron microscopy (Kaplan et al., 2000; Shao et al., 2019).

**Table 1.** Principal Actinopathies, Genes, and Structural/Histopathological Correlates

| **Disorder** | **Gene** | **Affected Tissue** | **Structural/Histopathological Correlate** |
|---|---|---|---|
| Baraitser-Winter syndrome | *ACTB, ACTG1* | Cerebral cortex, craniofacial tissues, face | Neuronal migration defects and cortical malformations, including pachygyria and lissencephaly (Di Donato et al., 2016; Rivière et al., 2012) |
| Nemaline myopathy | *ACTA1* | Skeletal muscle | Nemaline rods; intranuclear rods; cytoplasmic bodies and myofibrillar disorganization in muscle biopsy specimens (Labasse et al., 2022) |
| ACTC1-related cardiomyopathy | *ACTC1* | Cardiac muscle | Variant-dependent disruption of actin thin-filament interactions and contractile regulation; associated phenotypes include hypertrophic or dilated cardiomyopathy (Despond & Dawson, 2018) |
| Wiskott-Aldrich syndrome | *WAS* | Hematopoietic and immune cells | Microthrombocytopenia (hematological finding) and impaired actin reorganization during immune-synapse formation (Notarangelo & Ochs, 2003; Yarar et al., 1999) |
| DFNA20/26 hearing loss | *ACTG1* | Cochlear hair cells | Altered γ-actin function and disruption of actin-dependent stereociliary maintenance, associated with progressive hearing impairment (Morín et al., 2009) |
| ACTN4-related focal segmental glomerulosclerosis (FSGS) | *ACTN4* | Renal podocytes | Podocyte cytoskeletal dysfunction; renal biopsy may show segmental glomerulosclerosis and foot-process effacement (Kaplan et al., 2000; Shao et al., 2019) |

## 5. Clinical Manifestations and Diagnostic Tests

The structural and histopathological changes described above translate into clinical syndromes that are, for the most part, confined to the tissue in which the causative gene is predominantly expressed, and diagnosis increasingly relies on targeted or panel-based genetic testing rather than histopathology alone.

### 5.1 Baraitser-Winter Syndrome

Affected individuals present with a distinctive craniofacial gestalt (ptosis, broad nasal bridge, ridged metopic suture), ocular colobomata, and variable intellectual disability whose severity correlates loosely with the degree of cortical malformation seen on neuroimaging; epilepsy, sensorineural hearing loss, and skeletal anomalies are also common (Di Donato et al., 2016). Because ACTB haploinsufficiency produces a mechanistically and clinically distinct, generally milder disorder, accurate genetic characterization — distinguishing dominant-negative missense variants from loss-of-function variants — is clinically important for prognostic counseling (Cuvertino et al., 2017). An unexpected recent finding is that β-actin deficiency also disrupts T-cell function and immune regulation in Baraitser-Winter syndrome type 1, raising the possibility of an underrecognized immunological component to the disorder that may be amenable to targeted immunomodulatory therapy (Bar-On et al., 2025).

### 5.2 ACTA1-Related Nemaline Myopathy

Clinical severity spans a wide spectrum. At the severe end, infants present with profound congenital hypotonia ("floppy infant"), poor spontaneous movement, and respiratory insufficiency requiring permanent ventilatory support, with many affected infants dying within the first months of life; milder, later-onset forms present with slowly progressive proximal and axial weakness, feeding difficulty, and scoliosis (Labasse et al., 2022). Diagnosis relies on clinical hypotonia, characteristic muscle biopsy findings (nemaline rods on Gomori trichrome staining, confirmed ultrastructurally by electron microscopy), and genetic confirmation of an ACTA1 variant.

### 5.3 ACTC1-Related Cardiomyopathy

Patients with ACTC1-related hypertrophic cardiomyopathy may present with exertional dyspnea, chest pain, syncope, or through family screening, with risk of outflow obstruction and, in a subset, sudden cardiac death; those with dilated cardiomyopathy may present with heart-failure symptoms and systolic dysfunction on echocardiography.

A subset of patients present earlier in life with a congenital atrial-septal defect, sometimes recognized only incidentally, that later precedes the development of overt late-onset dilated cardiomyopathy, underscoring the value of longitudinal cardiac follow-up in ACTC1 variant carriers even when initial imaging is reassuring (Frank et al., 2019; Zarate et al., 2025).

### 5.4 Wiskott-Aldrich Syndrome

The classical clinical triad of Wiskott-Aldrich syndrome includes microthrombocytopenia with bleeding tendency, eczema, and recurrent sinopulmonary and other infections due to combined humoral and cellular immunodeficiency; over time, patients also face increased risk of autoimmune cytopenias and lymphoid malignancy. Clinical severity is graded using a standardized scoring system that guides the choice between conservative management and definitive, curative therapy such as hematopoietic stem cell transplantation or gene therapy (Ferrua et al., 2019).

### 5.5 DFNA20/26 Hearing Loss

Affected individuals typically have normal hearing at birth and develop progressive, bilateral, postlingual sensorineural hearing loss that begins in the high frequencies and, with advancing age, extends to all frequencies, consistent with the maintenance-dependent, rather than developmental, role of γ-actin in the stereocilium (Morín et al., 2009). Some ACTG1 variant carriers show phenotypic heterogeneity, including occasional non-syndromic presentations that overlap with other ACTG1-associated phenotypes, underscoring the value of molecular confirmation in atypical familial deafness (Sorrentino et al., 2021).

### 5.6 ACTN4-Related FSGS

Patients typically present in adolescence or adulthood with proteinuria that progresses toward nephrotic-range proteinuria and, ultimately, progressive chronic kidney disease; the autosomal dominant inheritance pattern and typically later age of onset distinguish ACTN4-related FSGS from most other genetic podocytopathies, which more often present in childhood. Diagnosis is supported by proteinuria, the clinical and family history, and identification of a pathogenic ACTN4 variant. When a renal biopsy is performed, segmental glomerulosclerosis and podocyte foot-process effacement can support the diagnosis; however, biopsy findings are not unique to ACTN4-related disease and renal biopsy should not be presented as an absolute prerequisite for molecular diagnosis (Grosch et al., 2025; Kaplan et al., 2000).

### 5.7 Genetic Diagnosis Across Actinopathies

Across the disorders discussed in this chapter, molecular testing is central to diagnosis and genotype–phenotype interpretation. Depending on the clinical presentation, family history, and local testing pathway, testing may use a targeted single-gene assay, a multigene panel, exome sequencing, or genome sequencing. These methods are genetic diagnostic tests rather than biomarkers. They are particularly useful when clinical presentations overlap with other inherited disorders and when variant class has prognostic implications, such as the distinction between ACTB altered-function missense variants and loss-of-function variants, or the characterization of ACTG1 variants in familial hearing loss (Di Donato et al., 2026; Wang et al., 2018).

## 6. Experimental Models

Because actin is essential for viability, modeling actinopathies has required approaches that introduce the specific, disease-causing variant rather than simply eliminating the gene. Patient-derived induced pluripotent stem cells, differentiated into cerebral organoids, have proven particularly valuable for Baraitser-Winter syndrome: conventional ACTB and ACTG knockout mice fail to reproduce the cortical malformations seen in patients, reflecting substantial differences between mouse and human cortical development, whereas organoids carrying the patient's own ACTB or ACTG1 missense variant recapitulate the reduced ventricular zone progenitor pool and diminished organoid growth observed in the human disease (Niehaus et al., 2026).

For nemaline myopathy, both transgenic zebrafish (TgACTA1-D286G) and mouse models (TgACTA1-D286G and knock-in KIActa1-H40Y) expressing dominant ACTA1 mutations reproduce skeletal muscle dysfunction and have served as the principal preclinical platforms for testing candidate therapies, including L-tyrosine supplementation, discussed further below (Messineo et al., 2018).

For ACTN4-related FSGS, podocyte-specific transgenic mice expressing mutant α-actinin-4 reproduce segmental glomerulosclerosis and proteinuria, establishing that podocyte-restricted expression of the mutant protein is sufficient to cause disease (Michaud et al., 2003), while more recent work has extended validation of newly identified ACTN4 variants into primary patient-derived fibroblasts and immortalized human podocyte cell lines, allowing variant pathogenicity to be confirmed at the cellular level before committing to animal studies (Grosch et al., 2025). For Wiskott-Aldrich syndrome, autologous CD34+ hematopoietic stem/progenitor cells transduced with a lentiviral WAS-encoding vector have served simultaneously as both a preclinical and a clinical platform,

since the same gene-corrected cell product used to demonstrate efficacy in early trials is the therapeutic product itself, discussed in the following section (Ferrua et al., 2019).

## 7. Current Therapeutic Approaches

Therapeutic strategies across the actinopathies remain heterogeneous, reflecting the diversity of affected tissues, and range from disease-modifying gene therapy to purely supportive, symptom-directed management.

Wiskott-Aldrich syndrome offers the most mature example of a disease-modifying approach. Autologous lentiviral hematopoietic stem/progenitor cell gene therapy, in which a patient's own CD34+ cells are transduced ex vivo with a WAS-encoding lentiviral vector and reinfused after reduced-intensity conditioning, produced stable gene-corrected cell engraftment with no serious treatment-related adverse events in an early-phase interim analysis, alongside resolution of severe infections and eczema and a significant reduction in autoimmune disease and bleeding episodes, despite only partial correction of the platelet compartment (Ferrua et al., 2019). Long-term follow-up at a median of 7.6 years confirmed durable safety and clinical benefit across multiple treated patients, establishing lentiviral gene therapy as a viable alternative to allogeneic hematopoietic stem cell transplantation, particularly for patients lacking a suitable donor (Magnani et al., 2022).

For nemaline myopathy, management remains predominantly supportive, including respiratory support, nutritional management, and physical therapy, because pharmacological correction of the underlying actin defect has proven difficult. A rigorously tested candidate, oral L-tyrosine supplementation, was evaluated across all three published dominant ACTA1 animal models (zebrafish and two independent mouse models) and produced no improvement in muscle function, strength, or histological integrity despite achieving adequate tissue drug levels, a negative result that has appropriately tempered clinical enthusiasm for this specific intervention and illustrates the broader difficulty of pharmacologically correcting a structural sarcomeric protein defect (Messineo et al., 2018).

For ACTC1-related cardiomyopathy, management follows standard heart-failure and hypertrophic cardiomyopathy guidelines (beta-blockers, other rate- and rhythm-controlling agents, implantable defibrillators in high-risk patients, and surgical or device-based intervention for outflow obstruction where indicated), since no actin-targeted pharmacological therapy is currently available; similarly, DFNA20/26 hearing loss is managed with conventional amplification

(hearing aids) and, in more advanced cases, cochlear implantation, rather than any disease-modifying intervention directed at the underlying actin defect.

For ACTN4-related FSGS, management follows general proteinuric kidney disease principles, including renin-angiotensin system blockade to reduce intraglomerular pressure, but a mechanistically specific therapeutic concept has emerged from structural studies: because EGF-stimulated phosphorylation of α-actinin-4 at tyrosine 4 and 31 reduces its pathologically excessive actin-binding affinity, pharmacological strategies that promote this phosphorylation, or that otherwise reduce mutant α-actinin-4's actin affinity, have been proposed as a rational, mutation-specific therapeutic approach, though this remains at the preclinical stage (Shao et al., 2019).

## 8. Future Perspectives

Several developments are likely to shape future work on actin cytoskeleton disorders. First, the success of lentiviral gene therapy in Wiskott-Aldrich syndrome provides a proof-of-concept template that may be adaptable, at least conceptually, to other monogenic actinopathies affecting accessible, transplantable cell populations, although the cerebral, cardiac, and renal actinopathies discussed in this chapter present considerably greater delivery challenges than the hematopoietic system (Ferrua et al., 2019; Magnani et al., 2022).

Second, large-scale genotype–phenotype correlation studies, such as recent multi-institutional efforts characterizing the full mutational and clinical spectrum of ACTB- and ACTG1-related non-muscle actinopathies, are refining the ability to predict disease severity and guide counseling from genotype alone, a capability that will become increasingly important as broader genomic sequencing identifies more variant carriers before overt clinical presentation (Di Donato et al., 2026).

Third, human cerebral organoid models are likely to be extended beyond Baraitser-Winter syndrome to other developmental actinopathies, offering a tractable platform for both mechanistic study and drug screening that captures human-specific aspects of tissue development that mouse models have, in several instances discussed in this chapter, failed to reproduce (Niehaus et al., 2026).

Finally, the negative result of L-tyrosine supplementation in nemaline myopathy models is a useful reminder that mechanistically plausible interventions require rigorous preclinical testing before clinical translation, and future therapeutic development for the muscle, cardiac, and renal actinopathies will likely need to move toward mutation-specific strategies, such as the α-actinin-4 phosphorylation concept proposed for FSGS, rather than generic supplementation approaches, paralleling the broader shift toward precision

genetic medicine across the actinopathies as a group (Messineo et al., 2018; Shao et al., 2019).

## 9. Conclusions

Actin cytoskeleton disorders demonstrate, with unusual clarity, how a single, evolutionarily ancient molecular system can be redeployed across radically different tissues and how its disruption produces a correspondingly diverse set of human diseases. Whether through dominant-negative ACTB/ACTG1 mutations that impair cortical progenitor proliferation, ACTA1 mutations that cause actin misfolding and aggregation in skeletal muscle, ACTC1 mutations that alter thin-filament calcium sensitivity in the heart, WASP loss-of-function that abolishes actin nucleation across the hematopoietic system, ACTG1 mutations that undermine long-term stereociliary maintenance in the cochlea, or ACTN4 mutations that pathologically rigidify the podocyte cytoskeleton, each actinopathy traces back to the same handful of conserved molecular operations: filament nucleation, polymerization, cross-linking, and turnover. These molecular defects produce tissue-specific, often diagnostic histopathological changes, translate into clinically distinct syndromes, and are increasingly characterized through precise genotype–phenotype correlation and genetic biomarker testing. Experimental models—from patient-derived cerebral organoids to transgenic zebrafish and podocyte-specific mice, have been essential to establishing these mechanisms, and therapeutic progress, most notably the durable success of lentiviral gene therapy in Wiskott-Aldrich syndrome, shows that disease-modifying treatment of an actinopathy is achievable when the affected cell population is accessible for genetic correction. Taken together, the evidence supports viewing the actin cytoskeleton not merely as a structural scaffold but as a common, mechanistically coherent axis along which a substantial and still-expanding set of human genetic diseases can be understood and, in some cases, treated.

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# Chapter 2

## Intermediate Filament Disorders: Tissue Integrity and Disease

**Gulce Naz Yazici**[1*]

**Abstract**

Intermediate filaments (IFs) form the third cytoskeletal system, and their tissue-specific proteins, keratins in the epidermis, desmin in striated and cardiac muscle, glial fibrillary acidic protein in astrocytes, and lamins at the nuclear envelope, underlie four clinically distinct disease families: the keratinopathies, the desminopathies, Alexander disease, and the laminopathies. This chapter reviews the normal histology, embryological origin, and molecular assembly of these networks, then surveys the histopathological and mechanistic basis of disease in each family, their clinical presentation and diagnostic markers, available experimental models, current therapeutic approaches, and the field's open questions. Across the four families, disease follows one organising principle rather than four unrelated ones: a network fails either mechanically, when a weakened filament cannot bear its tissue's load, or by aggregation into inclusions that are themselves the lesion. Neither mechanism is purely structural, since signalling is altered downstream of the lesion in more than one family; a phase 3 trial built on that premise in *LMNA*-related cardiomyopathy was negative, whereas the recent regulatory approvals of lonafarnib (progeroid laminopathies) and zilganersen (Alexander disease) show that disease-directed treatment is now possible in two of the four families. For histology and embryology, the four families converge on a single lesson: cytoskeletal identity is not incidental to tissue function, and correct assembly of a tissue-specific filament network during development is what its structural integrity depends on for life.

**Keywords:** intermediate filaments; cytoskeleton; keratinopathy; desminopathy; Alexander disease; laminopathy

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## 1. Introduction

Intermediate filaments (IFs) are the third filament system of the cytoskeleton, alongside actin microfilaments and microtubules, distinguished by their diameter (roughly 10 nm), a non-polar, nucleotide-independent assembly mechanism, and a large encoding gene family, 70 genes, giving 73 protein products once splice variants are counted, grouped into six types (Herrmann & Aebi, 2016; Omary, 2009; Szeverenyi et al., 2008). Unlike actin and tubulin, no single IF protein serves the whole body: each major tissue relies on its own designated member, keratins in epithelia, desmin in striated and cardiac muscle, glial fibrillary acidic protein (GFAP) in astrocytes, and the lamins in the nuclear envelope of essentially every nucleated cell.

This tissue-specific expression is why mutations in different IF genes produce clinically distinct diseases rather than one shared syndrome: a basal keratin mutation blisters the skin after mechanical trauma (Bolling et al., 2011); a desmin mutation damages striated and cardiac muscle through aggregation at the Z-disc (Claeys et al., 2009; Dalakas et al., 2000); a *GFAP* mutation produces the Rosenthal-fibre inclusions of Alexander disease (Brenner et al., 2001; Sosunov et al., 2017); and a lamin A/C (*LMNA*) mutation disrupts nuclear architecture rather than the cytoplasmic network — its cardiac phenotype the subject of a phase 3 trial of a targeted drug that did not meet its primary endpoint (Garcia-Pavia et al., 2024). What these families share, developed at length in Section 3, is not a common lesion but a common principle: an IF network is only as useful as its tissue-specific anchoring, and disease follows wherever that anchoring fails.

This chapter examines the four families- the keratinopathies, the desminopathies, Alexander disease, and the laminopathies- as a single group defined by shared structural logic rather than organ system, covering normal histology and embryological development (Section 2), molecular structure and disease mechanism (Section 3), clinical presentation and diagnostic markers (Section 4), experimental models (Section 5), therapeutic approaches (Section 6), and open questions (Section 7).

## 2. Normal Histology, Embryological Development, and Molecular Structure

A cell's shape, mechanical resistance to stress, capacity for directed intracellular transport, and ability to divide all depend on an internal scaffold, not the plasma membrane alone: the cytoskeleton. Three filament systems build this scaffold, each specialised for a different job: actin microfilaments generate contractile force and drive cell motility; microtubules provide tracks for organelle transport and form the mitotic spindle; and intermediate filaments, the system this chapter is about, provide

primarily mechanical, load-bearing support (Ross & Pawlina, 2016). IFs take their name from their diameter, roughly 10 nm, between the other two (Herrmann & Aebi, 2016). Unlike actin and microtubules, IFs are non-polar, assemble without consuming nucleotides, and are encoded not by a single conserved subunit but by a large family: 70 genes, 73 protein products once splice variants are counted, divided into six types: I–IV in the cytoplasm, V (the lamins) in the nucleus, and VI in the lens (Omary, 2009; Szeverenyi et al., 2008).

All IF proteins share the same building plan (Figure 1A): a central α-helical rod domain, flanked by variable head and tail domains, pairs into a coiled-coil dimer; two dimers associate antiparallel into a tetramer; tetramers then join end-to-end and side-to-side, compacting radially to the mature filament (Herrmann & Aebi, 2016). The antiparallel tetramer leaves the finished filament with no distinguishable ends, so IFs support no directed motor transport; their role is mechanical, not logistical. Single reconstituted filaments stretch to more than twice their resting length before breaking (Kreplak et al., 2008), a combination of extensibility and toughness that tissues exploit.

What differs between tissues is not the assembly mechanism but which protein is expressed, and where in the cell it is anchored (Figure 1B). The four tissues below illustrate what that means in practice: the same coiled-coil, non-polar building block is expressed as a different, tissue-restricted protein and anchored to a different structure in each, which turns one shared cytoskeletal system into four clinically distinct points of failure (Section 3).

**Epidermis.** The epidermis is keratinised stratified squamous epithelium, and its epithelial cells are called keratinocytes for this defining feature. Their intermediate filament cytoskeleton is built entirely from keratins, an obligate heteropolymer of one type I and one type II partner, and the expressed pair marks the cell's position within the epithelium (Ross & Pawlina, 2016). The epidermis arises from surface ectoderm, whose basal keratins 8 and 18 (K8/K18) are replaced, as the basal layer forms, by keratins 5 and 14 (K5/K14) with keratins 1 and 10 (K1/K10) in the differentiated layers above (Matsui, 2023). In mature skin, this keratinised stratified squamous epithelium continuously renews from stem cells in the stratum basale, and the K5/K14 → K1/K10 switch tracks cells from the basal to the spinous layer. Keratin bundles anchor into desmosomes laterally and hemidesmosomes basally, making one cell's network mechanically continuous with its neighbours and the basal lamina; in the granular layer, filaggrin and trichohyalin from keratohyalin granules aggregate these bundles as the cell cornifies (Ross & Pawlina, 2016).

**Striated muscle.** Skeletal muscle fibres form when myoblasts, derived from myogenic stem cells of the paraxial mesoderm and somites, fuse into myotubes, with satellite cells persisting beneath the external lamina for later regeneration. In

the mature fibre, desmin helps hold adjacent myofibrils' Z lines in register and anchors the sarcomere to the sarcolemma; in cardiac muscle the equivalent attachment site is the intercalated disc (Ross & Pawlina, 2016).

**Astrocytes.** Astrocytes derive from the neural tube. Both morphological types, protoplasmic (grey matter) and fibrous (white matter), contain bundles of GFAP, far more numerous in the fibrous type and the source of its name; GFAP antibodies routinely identify astrocytes in tissue sections (Ross & Pawlina, 2016).

**Nucleus.** The nuclear lamina, a thin IF meshwork against the inner nuclear membrane, composed principally of lamins A and C cross-linked into an orthogonal lattice and attached via lamin B and its receptors, including emerin, disassembles at the onset of mitosis and reassembles as the daughter nuclei form, unlike cytoplasmic IFs (Ross & Pawlina, 2016).

Across all four tissues, an IF network is only as useful as its attachments: desmosomes and hemidesmosomes, Z lines and the sarcolemma, and the inner nuclear membrane, which convert a filament inside one cell into mechanical continuity across a tissue. This is where IF disease begins.

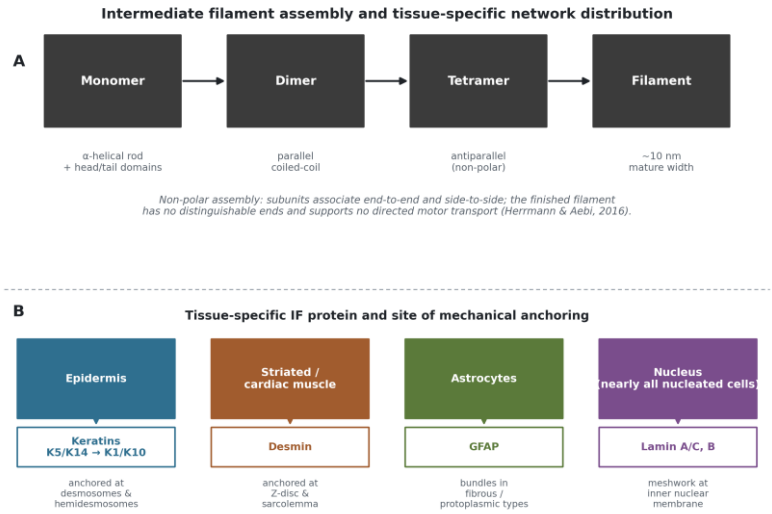



**Figure 1.** Intermediate filament (IF) assembly and tissue-specific network distribution. (A) Monomers assemble through a shared, non-polar mechanism, dimer, antiparallel tetramer, mature filament of about 10 nm. (B) Each of the four tissues expresses its own IF protein, anchored at a different structure: keratins at desmosomes and hemidesmosomes, desmin at the Z-disc and sarcolemma, GFAP as bundles in astrocytes, and lamins as a meshwork at the inner nuclear membrane. Disease in each of the four families follows from disruption of the tissue-specific network shown here (see Figure 2 and Section

3). Sources: Herrmann and Aebi (2016); Omary (2009); Szeverenyi et al. (2008); Ross and Pawlina (2016). Original schematic created by the author using Python (Matplotlib); the figure code was written with AI assistance (Claude, Anthropic). Not reproduced from any published figure.

## 3. Disease Mechanisms and Histopathological Changes

Intermediate filaments cause disease in two, not mutually exclusive, ways: a mutation may weaken the network so cells rupture under mechanical load, or make the protein aggregate into inclusions that are themselves the histopathological hallmark (Omary, 2009). Which tissue fails, and what the lesion looks like, follows from where the mutated protein is normally expressed.

### Keratinopathies: the lesion sits where the keratin does

Because keratins are the epidermis's principal load-bearing scaffold, a mutation here is felt almost immediately: clinically, as blistering; histologically, as separation at exactly the layer where the mutated keratin is expressed. Epidermolysis bullosa simplex (EBS) results from cleavage through basal keratinocytes after minor trauma; its prevalence is estimated at 1 in 25,000–50,000, making it the most common inherited blistering disease, a figure Bolling et al. (2011) quote from earlier sources rather than measure. In the largest tissue-confirmed series, 41 different mutations in *KRT5* and *KRT14* (the genes for keratins 5 and 14) were identified in unrelated, clinically characterised probands; 30 of them were heterozygous missense mutations, that is, single amino-acid substitutions, and the severe Dowling-Meara subtype showed tonofilament clumping in basal and suprabasal cells on electron microscopy (Bolling et al., 2011). The same lesion appears in the rarer recessive, localised form: splits within basal keratinocytes with keratin clumping confined mainly to that layer (Chen et al., 2021).

Move the mutation to a keratin expressed one layer higher, and the plane of damage moves with it: in epidermolytic hyperkeratosis, suprabasal *KRT1*/*KRT10* are mutated, and the network collapses in spinous and granular cells, with acanthosis and marked stratum corneum thickening. With age, blistering diminishes as hyperkeratosis dominates (Rothnagel et al., 1992).

### Desminopathies: a disease of the Z-disc, not of the whole myofibril

Desminopathy results from mutations in *DES*, the gene encoding desmin, the intermediate filament that anchors the muscle sarcomere; a *DES* mutation is its defining cause, though, as the discussion below on a chaperone protein shows, a mutation elsewhere can produce the same histological picture without a *DES*

mutation. Clinically, this Z-disc failure is also why desminopathy so often presents with both skeletal-muscle weakness and cardiac conduction disease or cardiomyopathy: the same protein anchors the same kind of structure in both tissues. In a cohort of 24 genetically characterised myofibrillar myopathy patients, 12 with *DES* mutations, the discriminating findings came from oxidative enzyme histochemistry: core-like lesions and 'rubbed-out' fibres, regions devoid of oxidative enzyme activity, usually in type 1 fibres, neither sharply demarcated nor confined to the subsarcolemmal zone, were constant in the desminopathies. Immunohistochemistry narrowed the mechanism further, targeting the specific proteins that make up the Z-disc's stress-sensing machinery, the logical candidates for what a Z-disc protein disease should disrupt: filamin C, myotilin and Xin were significantly mislocalised and aggregated, while α-actinin, myopodin, tritopodin and M-band proteins remained normal (Claeys et al., 2009). On electron microscopy, not performed in that cohort, a separate, independently ascertained series of 24 clinically diagnosed patients, 12 with confirmed *DES* mutations, showed the aggregated material itself to be amorphous and non-filamentous, with streaming Z-bands, rather than a compacted mass of intact desmin filaments (Dalakas et al., 2000). In practical terms, this means myofibrillar myopathy does not indiscriminately damage the muscle fibre: it targets a small, specific set of proteins that hold the Z-disc together under mechanical stress, while leaving the rest of the contractile machinery intact.

**Alexander disease: the inclusion is the diagnosis**

GFAP is the principal intermediate filament protein of mature astrocytes, and Alexander disease illustrates this chapter's second mechanism directly: the mutant protein does not merely fail to support the cell; it accumulates within the astrocyte itself as the pathological inclusion that gives the disease its name, a finding that doubles as its own diagnostic clue. Rosenthal fibres are the pathological hallmark of every form of the disease, caused by heterozygous, mostly non-conservative *GFAP* mutations; the clinical spectrum is in Section 4 (Brenner et al., 2001). Mouse models show the inclusions arising as granular deposits on intermediate filament bundles, containing GFAP and the small heat-shock protein αB-crystallin, that coalesce into the fibres seen by light microscopy; astrocytes carrying many lose their fine distal processes and acquire large, lobulated nuclei suggesting arrested mitosis (Sosunov et al., 2017).

**Laminopathies: the lesion is nuclear, and muscle histology is unhelpful**

The lamin meshwork underlies the nuclear envelope of essentially every nucleated cell, not one tissue-restricted cell type, which is why the same *LMNA* mutation can present as heart, skeletal-muscle, or neuromuscular-junction disease depending on which tissue's mechanical stress the nucleus can no longer withstand, and also why, among the four families here, tissue histology is usually of limited diagnostic help. Because lamins build a meshwork against the inner nuclear membrane, not a cytoplasmic network, *LMNA* disease damages nuclear architecture rather than the myofibril, and muscle biopsy is correspondingly non-specific (Maggi et al., 2021), diagnosis rests on genetics, not morphology. The same *LMNA* mutations can cause dilated cardiomyopathy type 1A, Emery-Dreifuss muscular dystrophy, or limb-girdle muscular dystrophy type 1B, while X-linked Emery-Dreifuss disease arises from mutations in emerin, a lamin receptor (Omary, 2009; Ross & Pawlina, 2016).

**Two threads run across the four families**

Two patterns cut across the disease-by-disease picture above. First, morphology does not identify the gene: 'rubbed-out' fibres are as characteristic of αB-crystallinopathy as of desminopathy (Claeys et al., 2009) (Figure 2). The tissue looks the same whether the broken part is the filament itself or the machinery that keeps it soluble, and αB-crystallin is not an intermediate filament protein but a chaperone that is also a component of the Rosenthal fibres of Alexander disease (Sosunov et al., 2017), failure of the machinery that keeps filaments soluble reproduces the histology of a mutation in the filament itself. Second, none of these is purely mechanical diseases, not even the ones that look purely structural: in recessive EBS the mutant network came with inhibited MAPK signalling and upregulated desmoglein 1 (Chen et al., 2021), and in *LMNA*-related cardiomyopathy mitogen-activated protein kinase (MAPK) signalling became the basis of a phase 3 trial (Garcia-Pavia et al., 2024), a trial whose negative result, discussed in Section 6, leaves open whether this signalling link can be used therapeutically.

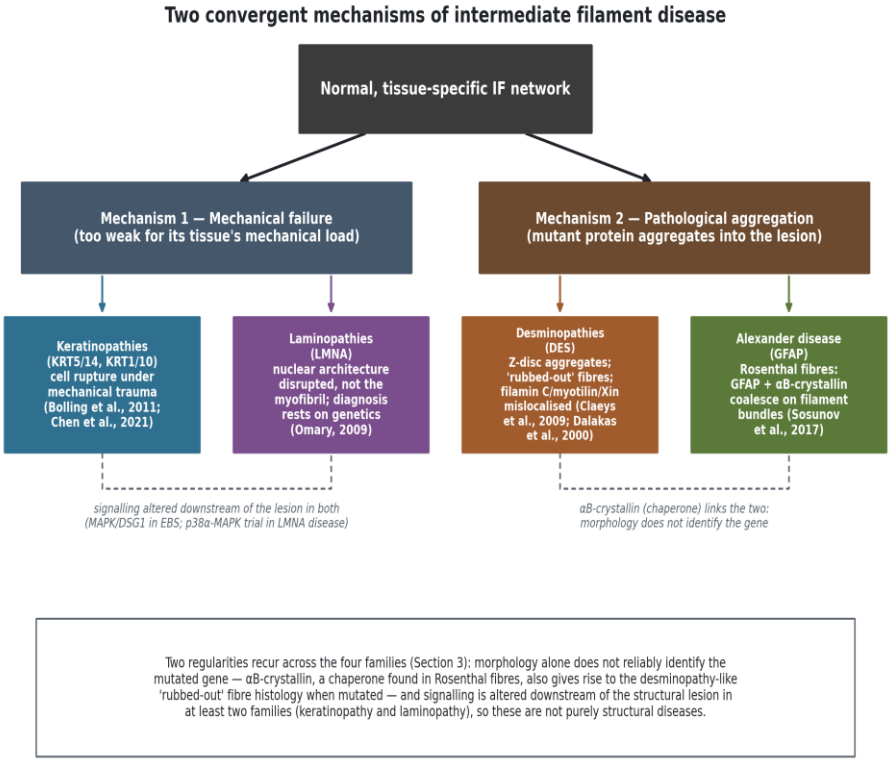



**Figure 2.** The four intermediate filament disease families converge on two mechanisms, mechanical failure or pathological aggregation, rather than four independent ones. Placement under each mechanism reflects the account given in Section 3. Dashed brackets mark two regularities that cut across this division: αB-crystallin, a chaperone and component of Rosenthal fibres, produces desminopathy-like histology when mutated, so morphology does not identify the gene; and signalling is altered downstream of the structural lesion in at least two families (keratinopathy and laminopathy). Original schematic created by the author using Python (Matplotlib); the figure code was written with AI assistance (Claude, Anthropic). Not reproduced from any published figure.

### 4. Clinical Manifestations and Diagnostic Markers

Which tissue carries the mutated protein determines what a patient actually experiences: the histology described in Section 3 becomes, at the bedside, a distinct set of symptoms for each of the four families. In keratinopathies, the phenotype is mechanical skin fragility: blistering after minor trauma, generalised from early infancy in severe subtypes, and limited to the hands and

feet in the localised form (Bolling et al., 2011). In epidermolytic hyperkeratosis, blistering gives way to hyperkeratosis with age; the presenting complaint changes over a patient's lifetime (Rothnagel et al., 1992).

Muscle and heart dominate the desminopathies. Pooled data from 159 genetically confirmed *DES* carriers across 39 reports show neurological and cardiological signs each in 74% (both together in 49%); cardiomyopathy in 49%, dilated 17%, restrictive 12%, hypertrophic 6%, arrhythmogenic right ventricular 1%, and conduction disease/arrhythmia in 62%, death in 26%. Genotype tracks phenotype by domain: 2B rod-domain mutations predominate in isolated neurological disease (90%), head- or tail-domain mutations in isolated cardiological disease (65%) (van Spaendonck-Zwarts et al., 2011). Two independent single-centre cohorts converge on a high cardiac-event burden: in 28 French carriers followed a mean 10 years, cardiac involvement occurred in 27 and a major adverse cardiac event usually a high-degree conduction defect needing a pacemaker in 13, with five deaths and no mutation-site/phenotype association (Wahbi et al., 2012); in a 53-patient Spanish myofibrillar myopathy cohort, cardiomyopathy developed in 16 of 18 *DES* carriers, against 1 of 24 with myotilin and 1 of 7 with mutations in ZASP, another Z-disc protein gene, causing its own, genetically distinct myofibrillar myopathy, presenting roughly three decades earlier (mean 28 years) (Olivé et al., 2011). Against a defined screening population rather than a myopathy case series, *DES* mutations accounted for only 1.4% of dilated cardiomyopathy among 425 screened probands, one of the field's few prevalence figures with a stated denominator (Taylor et al., 2007).

The population assembled for the field's one phase 3 trial gives a concrete picture of advanced *LMNA*-related cardiac disease: adults in New York Heart Association (NYHA) class II–III — heart failure already causing symptoms such as fatigue or breathlessness with mild-to-moderate exertion, ejection fraction ≤50%, with an implanted defibrillator (trial design and outcome in Section 6; Garcia-Pavia et al., 2024).

In Alexander disease, the infantile form presents with macrocephaly, seizures and psychomotor retardation, usually fatal within the first decade; juvenile and adult forms present with ataxia, bulbar signs and spasticity on a slower course (Brenner et al., 2001).

Diagnosis rests on genetics, supported rather than replaced by morphology, and the yield is imperfect: *KRT5*/*KRT14* mutations were identified in 57 of 76 unrelated probands with biopsy-confirmed EBS, leaving a quarter genetically unexplained (Bolling et al., 2011). The useful tissue markers identify lesions, not diseases. In myofibrillar myopathy, aggregates are desmin-immunoreactive

and mislocalize filamin C, myotilin, and Xin, while α-actinin and M-band proteins remain normal (Claeys et al., 2009); on electron microscopy, the aggregated material is amorphous and non-filamentous, with streaming Z-bands rather than intact filaments (Dalakas et al., 2000). Rosenthal fibres contain GFAP and αB-crystallin and are reliably labelled with the nuclear dye 4′,6-diamidino-2-phenylindole (DAPI), which can be combined with immunolabelling (Sosunov et al., 2017).

None of the sources consulted reports a validated immunohistochemical panel, with sensitivity and specificity figures, for any of the four families. The most advanced IF-protein biomarker, however, is not from any of these four families: neurofilament light chain (NfL), a blood marker released whenever a nerve fibre is damaged, for almost any reason. NfL has already reached the clinic once; it helped win accelerated U.S. Food and Drug Administration (FDA) approval for tofersen, a drug for a different genetic disease, SOD1-associated amyotrophic lateral sclerosis (SOD1-ALS), a real milestone for the IF biomarker field in general. But that is also its limitation: NfL rises with nerve damage broadly rather than with any one disease specifically, and it is not established as a biomarker for any of the four families discussed in this chapter (Khalil et al., 2024).

## 5. Experimental Models

Experimental models of intermediate filament disease fall into three tiers that answer different questions; in this field, a model's informative feature is often the built-in control, not the mutant phenotype itself. Each tier answers a different question about how a mutation causes disease: does the filament itself become mechanically weaker, does a whole tissue lose its integrity, or does a human cell carrying the patient's own mutation behave abnormally? The three levels below are ordered accordingly, from an isolated filament to mouse tissue to a patient-derived human cell.

At the lowest level of organisation, single filaments assembled in vitro can be loaded directly: pushing laterally on reconstituted murine desmin filaments with an atomic force microscope tip produced extensions of 8–240% across 60 events, mean 100%, with pronounced strain-hardening at high extension (Kreplak et al., 2008). Such measurements are method-dependent; tensile strength from the same data is 240–420 MPa, megapascals, the force per unit area the filament can bear before it fails, depending on the geometric model applied. A review of atomic force measurements of cellular stiffness reports 0.4–27 kPa, the same kind of unit but here describing how stiff, rather than how strong, the probed surface is, a spread following tip geometry, contact area,

indentation speed, passage number and cell type rather than biology (Sapra & Medalia, 2021); values from different platforms should not be compared numerically.

In animal models, the design carries the argument: conditional keratin 8 deletion in the intestinal epithelium disrupts tissue integrity and predisposes the colon to tumourigenesis, with the same study comparing single-allele animals for an internal gene-dose control (Stenvall et al., 2022); the tumour phenotype is carcinogen-dependent, not spontaneous.

Patient-derived pluripotent stem cells now supply the tissue-level models the classical families lacked: skin, heart or brain cells grown from a patient's own reprogrammed cells, carrying that patient's exact mutation, increasingly with an isogenic gene-corrected control, the same cell line, with only the mutation repaired, so any difference observed can be attributed to that one change, rather than an unrelated healthy line. Three of the four families now have such a model. For Alexander disease, neural/cortical organoids from induced pluripotent stem cells (iPSCs) bearing GFAP R239C, versus their corrected isogenic control, show altered lineage commitment and reduced astrocyte abundance (Matusova et al., 2025). For desminopathies, human iPSC-derived cardiomyocytes carrying the desmin variant E439K reproduce desmin aggregation with mitochondrial and sarcomeric defects, and three-dimensional engineered heart tissue built from them shows markedly reduced contractile force (Batoumeni et al., 2025). A related but simpler model exists for the laminopathies: iPSC-derived cardiomyocytes from a family carrying *LMNA* c.357-2A>G, engineered into anisotropic cardiac tissues on micropatterned planar substrates, generated significantly weaker systolic and active stress than control tissues (two patient and three control lines; Mehrabi et al., 2021), a micropatterned two-dimensional culture, not a 3D construct. The keratinopathies remain furthest behind: keratinocytes derived from the iPSCs of three patients with *KRT5* variants reproduce keratin aggregates, reduced proliferation and altered ERK signalling in culture (Coutier et al., 2022), but tissue-level models reproducing basal keratinocyte rupture are so far reported only in abstract form.

## 6. Current Therapeutic Approaches

For most of the classical intermediate filament disorders, the treatment available today addresses the consequences of the disease, not its cause. Management of blistering keratinopathies remains supportive, with wound care and infection control; for the muscle families, a recent review describes management centred on cardiac rhythm surveillance, device therapy, and

standard heart-failure treatment, with no causative option available (Maggi et al., 2021). Two recent regulatory approvals show this is no longer uniformly true, one for each family.

In September 2026 the U.S. Food and Drug Administration (FDA) approved zilganersen (Zanvastro) for Alexander disease and described it as the first drug for this condition. The approval rested on a randomised controlled study (NCT04849741) of 49 patients aged 2 years or older, in which patients aged 5 years and older with measurable walking difficulty at baseline showed significantly better walking speed at 61 weeks than untreated patients (U.S. Food and Drug Administration, 2026). A peer-reviewed report of that study was not available at the time of writing, so we could not independently appraise its design and effect size here. In November 2020 the FDA approved lonafarnib (Zokinvy) both to reduce the risk of mortality in Hutchinson–Gilford progeria syndrome and to treat processing-deficient progeroid laminopathies, defined as a heterozygous *LMNA* mutation with progerin-like protein accumulation or homozygous or compound heterozygous *ZMPSTE24* mutations (U.S. Food and Drug Administration, 2020).

Outside these two approvals, the phase 3 trial in cardiac laminopathies identified for this chapter did not succeed. REALM-DCM, a phase 3 trial in *LMNA*-related dilated cardiomyopathy (DCM), tested the selective p38α MAPK inhibitor ARRY-371797 (400 mg twice daily) against placebo in 77 patients with a confirmed *LMNA* variant, NYHA class II–III symptoms, ejection fraction ≤50%, an implanted defibrillator and reduced walking distance, across 31 sites in six countries. It was stopped early after a planned interim analysis indicated futility. Its primary outcome was the change in six-minute walk distance, assessed at 24 weeks after being moved from 12 weeks during the trial. The median change was 21 m on drug and 3 m on placebo, and the between-group difference was not significant (Hodges–Lehmann estimate 4,9 m; 95% CI −24.2 to 34.1; P = 0.82). The authors reported no new safety findings, although dose reductions (28% vs 8%), study discontinuation (28% vs 14%) and diarrhoea (30% vs 11%) were more frequent on drug (Garcia-Pavia et al., 2024).

It is worth asking why treatment has advanced further next door than it has here. The contrast with adjacent diseases is instructive: autologous transgenic epidermal stem cells restored a durable, blister-free epidermis in a child with junctional epidermolysis bullosa, a disease of the basement membrane anchoring the epidermis, not of the keratin filament itself (Hirsch et al., 2017); and an intrathecal adeno-associated virus (AAV) *GAN* gene transfer was given to 14 children with giant axonal neuropathy (GAN) in a dose-escalation trial with safety as its primary end point (Bharucha-Goebel et al., 2024), gigaxonin,

the gene involved, regulates intermediate filaments but is not itself one. Gene-directed treatment has thus already reached patients whose disorders damage the filament network only indirectly, while none of the sources reviewed for this chapter reports a therapy directed at the filament defect itself for desminopathies and keratinopathies.

## 7. Future Perspectives

Four problems in this field are practical rather than conceptual, and each has a concrete remedy. First, the numbers are method-dependent and few: filament diameter differs by imaging method and the filament population is not uniform (Herrmann & Aebi, 2016), and the extensibility figures quoted throughout the literature trace to a single set of 60 atomic-force stretching events on reconstituted desmin (Kreplak et al., 2008). Reporting standards that require platform, geometry, and sample number alongside every mechanical value would improve comparability more than additional measurements. Second, the field's descriptive statistics are dated database snapshots, not findings: the 70 encoding genes, 73 protein products, and disease count all derive from a curated database whose totals rise with each revision (Szeverenyi et al., 2008; Omary, 2009). Counts should be cited with a version and access date. Third, patient-derived three-dimensional models arrived only recently, each representing a single mutation, with isogenic gene-corrected controls becoming the standard comparator (Matusova et al., 2025); tissue-level keratin-disease models remain the least developed. Fourth, morphology does not identify the gene: identical lesions arise from mutation of a filament or of the chaperone that keeps it soluble (Claeys et al., 2009) (Figure 2). Quantitative morphometry and proteomic analysis of the aggregates, rather than further descriptive series, will separate them.

## 8. Conclusions

Read together, the four families are not four separate stories but one: a filament network fails either because it is mechanically too weak for the load its tissue places on it, or because the mutant protein aggregates into the inclusion that is itself the disease's hallmark lesion and, within a single family, these are not mutually exclusive (Omary, 2009). Neither mechanism is purely structural: in more than one family, the lesion also disrupts downstream signalling, a link the phase 3 trial built on that premise, which failed, did not validate (Chen et al., 2021; Garcia-Pavia et al., 2024). None of this should be read as more settled than it is. Section 7 sets out where this evidence base is still thin, in measurement, in models, in biomarkers, and that accounting is not repeated

here. What the four families share, in the end, is a lesson that belongs specifically to histology and embryology: an intermediate filament network is not a passive filler of the cytoplasm, but an actively built, tissue-specific structure, and even a single amino-acid substitution in it can be enough to compromise the tissue that depends on it (Section 3). Closing the gaps this chapter has identified, in measurement, in models, in biomarkers, is what will turn today's descriptive account of these four diseases into a mechanistic one.

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# Chapter 3

## Motor Protein Defects: Dynein, Kinesin and Myosin-Associated Diseases

**Ahmet Cabir[1*], Feyzullah Beyaz [2]**

**Abstract**

Motor proteins are ATP-dependent molecular machines that generate force and directed movement along cytoskeletal filaments. They play essential roles in the spatial organization of eukaryotic cells by transporting a wide variety of cellular components, including vesicles, organelles, proteins, and RNA-containing particles. The three major families of cytoskeletal motor proteins are myosins, kinesins, and dyneins. The directionality and functional specialization of motor proteins enable the controlled distribution of cargo within the cell. Most kinesins mediate transport toward the plus ends of microtubules, whereas cytoplasmic dynein is the principal motor responsible for minus-end-directed transport. Myosins perform diverse functions along actin filaments, ranging from force generation and muscle contraction to the short-range transport and positioning of intracellular cargo. The importance of motor-driven intracellular transport is particularly evident in highly polarized cells such as neurons, where proteins, membrane-bound vesicles, organelles, and other cellular components must be transported over exceptionally long distances. Alterations in molecular motors, motor–cargo interactions, cytoskeletal organization, or transport regulatory mechanisms can disrupt the spatial and temporal distribution of cellular components, contributing to neuronal dysfunction and neurodegenerative diseases. Consequently, understanding intracellular transport mechanisms provides an essential basis for elucidating how abnormalities in motor proteins and their associated transport machinery contribute to disease pathogenesis.

**Keywords:** Motor proteins, motor protein defects, dynein, kinesin, myosin

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## 1. Introduction

Motor proteins are ATP-dependent molecular machines that convert chemical energy into mechanical force, supporting intracellular transport, cellular organization, and motility. The three major families kinesins, dyneins, and myosins operate along cytoskeletal filaments to transport and position vesicles, organelles, protein complexes, and RNA-containing particles. Kinesins and dyneins primarily move along microtubules, whereas myosins function along actin filaments. Together, these motors contribute to essential cellular processes, including membrane trafficking, organelle distribution, cell division, and cellular movement (Roberts et al., 2013; Sweeney and Holzbaur, 2018).

Efficient intracellular transport requires precise coordination between molecular motors, cytoskeletal tracks, cargo-specific adaptors, and regulatory proteins. This coordination enables cellular components to reach appropriate destinations and supports the maintenance of cellular homeostasis. The importance of these mechanisms is particularly evident in neurons, where the highly polarized architecture and considerable length of axons require continuous bidirectional transport between the cell body and distant synaptic compartments. Kinesin- and dynein-dependent transport supports the distribution of essential cargoes, while myosins contribute to localized trafficking and cargo positioning within actin-rich neuronal regions (Hirokawa et al., 2010; De Vos and Hafezparast, 2017).

Beyond intracellular transport, molecular motors perform specialized functions in structures such as motile cilia and inner ear stereocilia. Defects affecting motor proteins, their assembly, or their interactions with cargo and regulatory partners can therefore produce diverse pathological consequences. Impaired axonal transport contributes to neurological dysfunction, abnormalities in axonemal dynein can cause primary ciliary dyskinesia, and defects in specific myosins can disrupt stereocilia organization and lead to hereditary hearing loss. This review outlines the principal functions of motor proteins in intracellular transport, vesicle trafficking, and axonal transport, and examines the mechanisms linking their dysfunction to human disease.

### 1.1. Motor Proteins: Dynein, Kinesin and Myosin

Motor proteins are ATP-dependent molecular machines that generate force and directed movement along cytoskeletal filaments. They play essential roles in the spatial organization of eukaryotic cells by transporting a wide variety of cellular components, including vesicles, organelles, proteins, and RNA-containing particles. The three major families of cytoskeletal motor proteins are myosins, kinesins, and dyneins. Myosins primarily operate along actin filaments,

whereas kinesins and dyneins use microtubules as their tracks. Despite their distinct molecular architectures and mechanisms of force generation, these motors convert the chemical energy derived from ATP hydrolysis into mechanical work and thereby support fundamental cellular processes such as intracellular transport, membrane trafficking, organelle positioning, cell division, and cellular motility (Roberts et al., 2013; Sweeney and Holzbaur, 2018).

The directionality and functional specialisation of motor proteins enable the controlled distribution of cargo within the cell. Most kinesins mediate transport toward the plus ends of microtubules, whereas cytoplasmic dynein is the principal motor responsible for minus-end-directed transport. Myosins perform diverse functions along actin filaments, ranging from force generation and muscle contraction to the short-range transport and positioning of intracellular cargo. The coordinated activity of these motor systems is particularly important for vesicle trafficking and axonal transport, where cellular materials must be efficiently transported between distant intracellular compartments.

Cytoplasmic dynein, for example, transports membrane-bound organelles, vesicles, proteins, and RNA-containing particles toward microtubule minus ends, while kinesin motors generally support transport in the opposite direction. Disrupting these highly regulated transport mechanisms can impair cellular homeostasis and contribute to neurological, developmental, and other motor protein-associated diseases (Table 1) (Roberts et al., 2013; Sweeney and Holzbaur, 2018; Canty et al., 2021).

**Table 1.** The table summarises the characteristic features of the three major motor proteins: dynein, kinesin, and myosin.

| Feature | Kinesin | Dynein | Myosin |
|---|---|---|---|
| **Movement direction** | Toward the plus (+) end of microtubules (anterograde) | Toward the minus (−) end of microtubules (retrograde) | Usually toward the plus (+) end of actin filaments *(except Myosin VI)* |
| **Track** | Microtubules | Microtubules | Actin filaments |
| **ATPase activity** | ATP-dependent | ATP-dependent | ATP-dependent |
| **Major functions** | Intracellular transport, mitotic spindle assembly, chromosome segregation | Retrograde transport, ciliary and flagellar motility, spindle positioning | Muscle contraction, cytokinesis, cell migration, intracellular transport |

| | | | |
|---|---|---|---|
| **Representative members** | KIF5 (Kinesin-1), KIF3 (Kinesin-2), KIF11 (Eg5) | Cytoplasmic dynein, Axonemal dynein | Myosin I, II, V, VI |
| **Typical cargo** | Vesicles, mitochondria, lysosomes, mRNA, protein complexes | Endosomes, lysosomes, Golgi-derived vesicles, nuclei | Secretory vesicles, melanosomes, endoplasmic reticulum, actin-associated organelles *(isoform-dependent)* |
| **Major biological roles** | Axonal transport, organelle trafficking, mitosis | Retrograde axonal transport, ciliary beating, intracellular organization | Muscle contraction, cell motility, cytokinesis, membrane trafficking |

### 1.2. Intracellular Transport

Intracellular transport is a highly regulated process that ensures the spatial organization and distribution of cellular components. Eukaryotic cells contain a wide variety of cargoes, including membrane-bound organelles, vesicles, protein complexes, and mRNAs, which must be efficiently delivered to specific intracellular destinations. This transport system primarily depends on cytoskeletal filaments that serve as tracks for molecular motors. Microtubules support long-distance transport mediated mainly by kinesin and dynein motors, whereas actin filaments and myosin motors predominantly contribute to localized and short-range transport, particularly in actin-rich cellular regions. The coordinated activity of these transport systems contributes to the appropriate distribution and organization of intracellular components (Barlan and Gelfand, 2017; Hirokawa et al., 2010).

The polarised organisation of microtubules provides directional control of intracellular cargo movement. In most cell types, kinesins generally mediate transport toward microtubule plus ends and the cell periphery, whereas cytoplasmic dynein primarily drives transport toward minus ends located near the microtubule-organizing center. However, intracellular transport is more complex than the independent activity of individual motors, as opposing motor proteins can simultaneously associate with the same cargo. Interactions among molecular motors, cargo-specific adaptor proteins, scaffolding proteins, and other regulatory molecules regulate the direction, timing, and efficiency of cargo movement, allowing cells to modify organelle transport in response to physiological signals and changes in the intracellular environment (Fu and Holzbaur, 2014).

The importance of motor-driven intracellular transport is particularly evident in highly polarized cells such as neurons, where proteins, membrane-bound vesicles, organelles, and other cellular components must be transported over exceptionally long distances. Alterations in molecular motors, motor–cargo interactions, cytoskeletal organization, or transport regulatory mechanisms can disrupt the spatial and temporal distribution of cellular components, contributing to neuronal dysfunction and neurodegenerative diseases. Consequently, understanding intracellular transport mechanisms provides an essential basis for elucidating how abnormalities in motor proteins and their associated transport machinery contribute to disease pathogenesis (Figure 1) (De Vos and Hafezparast, 2017).

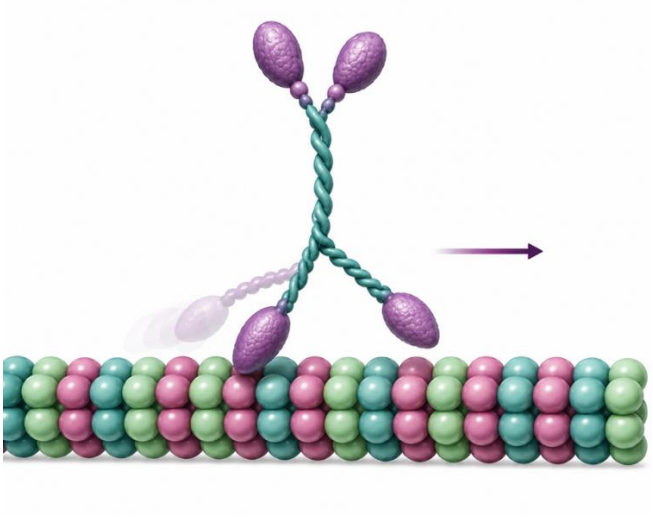


**Figure 1.** Schematic illustration of a motor protein in motion. Created by the authors on Adobe.

### 1.3. Vehicle Trafficking

Vesicle trafficking is a fundamental component of intracellular transport, moving proteins, lipids, and other cellular materials between intracellular compartments and the plasma membrane. Membrane-bound organelles, including the endoplasmic reticulum and Golgi apparatus, are major sites for processing, sorting, and vesicle formation. After formation, transport vesicles are directed to specific intracellular destinations, where they interact with and fuse with target membranes to deliver their cargo. Long-distance vesicle movement primarily depends on the coordinated activity of cytoskeletal tracks and molecular motor proteins (Caviston and Holzbaur, 2006; Qu et al., 2025).

Microtubules serve as the principal tracks for long-distance vesicle transport, whereas actin filament-based transport systems predominantly contribute to localized trafficking events. Kinesin motors generally transport vesicles toward microtubule plus ends, while cytoplasmic dynein mediates transport toward minus ends. Motor proteins are recruited to vesicular membranes through interactions involving cargo-specific adaptor proteins, scaffolding proteins, and regulatory molecules such as Rab GTPases. These interactions integrate membrane trafficking with motor-driven transport and contribute to accurate spatial and temporal cargo delivery (Caviston and Holzbaur, 2006; Fu and Holzbaur, 2014; Hirokawa et al., 2010).

Individual molecular motors exhibit functional specialization and contribute to the transport of distinct vesicular cargoes. For example, KIF1A and KIF1Bβ transport synaptic vesicle precursors, whereas KIF5 family members transport diverse neuronal cargoes, including mitochondria and vesicles containing amyloid precursor protein and neurotrophin receptors. Cytoplasmic dynein primarily supports retrograde transport, including the movement of signaling endosomes toward the neuronal cell body, while myosin motors contribute to localized vesicle transport within actin-rich cellular compartments. Such functional specialization enables the selective distribution of vesicular cargoes to appropriate intracellular destinations (Hirokawa et al., 2010).

Because individual vesicles can associate with molecular motors of opposing directionalities, efficient trafficking requires precise coordination of motor activity. Coordination between kinesin- and dynein-mediated movement enables bidirectional transport and supports appropriate vesicle distribution within the cell. Disruption of motor activity, motor–cargo interactions, or regulatory pathways can impair vesicle distribution and intracellular communication. Such abnormalities are particularly detrimental in neurons, where efficient vesicle trafficking is required for synaptic maintenance and neuronal homeostasis. Consequently, defects in motor-dependent vesicle trafficking are increasingly recognized as important contributors to neurological and neurodegenerative disorders (Figure 2) (Fu and Holzbaur, 2014; Anderson et al., 2014).

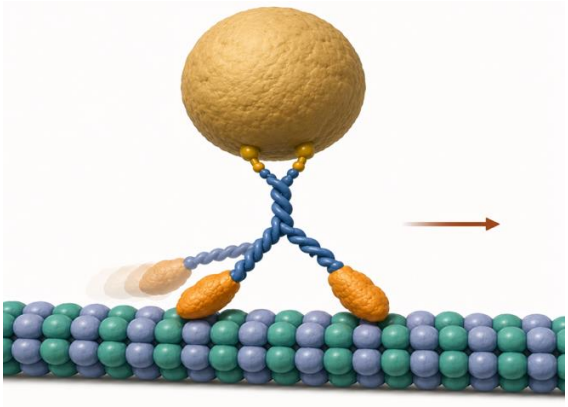


**Figure 2.** Schematic representation of a motor protein carrying its cargo. Created by the authors on Adobe.

### 1.4. Axonal Transport

Axonal transport is a specialized form of intracellular transport that enables the bidirectional movement of cellular components between the neuronal cell body and distant regions of the axon. Because neurons are highly polarized cells and axons may extend over exceptionally long distances, passive diffusion alone is insufficient to maintain neuronal function. Instead, axonal transport relies primarily on microtubule tracks and molecular motors to deliver a wide range of cargoes, including synaptic vesicle precursors, mitochondria, signaling endosomes, autophagosomes, lysosomes, and mRNA-containing granules. This transport system is essential for neuronal development, maintenance, synaptic function, and long-distance communication between the soma and axon terminals (Guedes-Dias and Holzbaur, 2019).

The polarized arrangement of axonal microtubules establishes the directionality of long-distance cargo transport. Kinesin motors primarily mediate anterograde transport toward distal axonal regions, whereas cytoplasmic dynein mediates retrograde transport toward the neuronal cell body. Axonal transport is commonly classified as fast or slow according to the rate and nature of cargo movement. Fast axonal transport mainly carries membrane-bound organelles and vesicles, whereas slow axonal transport primarily transports cytoskeletal elements and soluble protein complexes. Efficient transport requires coordinated interactions among molecular motors, adaptor proteins, regulatory molecules, and the microtubule cytoskeleton (Anderson et al., 2014; Guedes-Dias and Holzbaur, 2019). Although long-distance axonal transport primarily depends on kinesin and dynein motors, myosin motors contribute to short-range cargo transport and positioning within actin-rich neuronal compartments, including

presynaptic terminals, postsynaptic regions, and growth cones. The coordinated activities of microtubule- and actin-based motor systems therefore enable cargoes to be transported over long distances and subsequently positioned within specialized neuronal compartments (Hirokawa et al., 2010).

Disruption of axonal transport has profound consequences for neuronal survival because neurons rely on continuous bidirectional cargo trafficking to maintain their highly polarized architecture. Mutations affecting kinesin or dynein motors, abnormalities in motor–cargo interactions, alterations in microtubule organization, and defects in transport regulatory pathways can impair axonal transport and ultimately contribute to neuronal dysfunction. Increasing evidence indicates that impaired axonal transport is a common feature of numerous neurodegenerative disorders and that mutations affecting components of the axonal transport machinery can directly cause disease. Therefore, axonal transport represents one of the principal cellular mechanisms linking motor protein dysfunction to the pathogenesis of neurological disorders (De Vos and Hafezparast, 2017).

## 2. Motor Protein Defects

### 2.1. Primary Ciliary Dyskinesia

Primary ciliary dyskinesia (PCD) is a genetically heterogeneous disorder of motile cilia caused by abnormalities in proteins involved in ciliary assembly, structure, and function. Motile cilia are microtubule-based organelles that generate coordinated movement in several tissues, including the respiratory and reproductive tracts. Their axonemal structure typically consists of nine peripheral microtubule doublets surrounding a central pair of microtubules. Axonemal dyneins are ATP-dependent motor protein complexes attached to the peripheral microtubule doublets and generate the mechanical force required for ciliary beating. Through ATP hydrolysis, dynein-driven sliding between adjacent microtubule doublets is converted into coordinated bending of the ciliary axoneme. Consequently, abnormalities affecting axonemal dyneins or other components required for motile cilia function can impair ciliary motility and contribute to PCD (Antony et al., 2021; Despotes et al., 2024).

The relationship between dynein dysfunction and PCD is particularly important because axonemal dynein complexes constitute the principal motor machinery responsible for ciliary movement. These complexes are organized into outer and inner dynein arms, which contribute to the generation and regulation of ciliary beating. Pathogenic variants affecting axonemal dynein heavy chains, intermediate chains, light chains, or proteins involved in dynein arm assembly can disrupt the formation or function of these motor complexes. Such

abnormalities may result in absent, reduced, or dysregulated ciliary movement and compromise mucociliary clearance in the respiratory tract. However, PCD is genetically and structurally heterogeneous, and disease-causing variants may also affect other components involved in ciliary assembly and motility rather than the dynein motors themselves (Antony et al., 2021; Despotes et al., 2024).

Impaired motile cilia function produces a broad spectrum of clinical manifestations. Defective mucociliary clearance commonly results in neonatal respiratory distress and recurrent upper and lower respiratory tract infections, which may progressively contribute to chronic airway disease and bronchiectasis. Dysfunction of motile cilia in the reproductive system can cause subfertility or infertility, while abnormalities in embryonic nodal cilia can disrupt normal left–right body patterning and lead to laterality defects. The clinical presentation and severity of PCD vary considerably among affected individuals, reflecting the disease's underlying genetic and phenotypic heterogeneity (Despotes et al., 2024).

The molecular pathology of PCD shows a direct link between motor protein dysfunction and human disease. Defects in axonemal dynein complexes interfere with the generation of the mechanical forces required for normal ciliary movement. The resulting impairment of ciliary motility disrupts essential physiological processes, particularly mucociliary clearance, and contributes to progressive clinical complications. Thus, PCD is an important example of a motor protein-associated disease in which abnormalities in dynein-dependent motility directly link molecular motor dysfunction to tissue pathology and clinical manifestations (Antony et al., 2021).

### 2.2. Neurological Disorders

Neurological disorders represent an important group of diseases associated with molecular motor dysfunction. Neurons are particularly vulnerable to defects in motor proteins because their highly polarized morphology requires the continuous transport of proteins, organelles, vesicles, and signaling molecules over exceptionally long intracellular distances. Kinesin, cytoplasmic dynein, and myosin motors perform specialized and complementary functions within axons, dendrites, and synaptic compartments. Consequently, pathogenic alterations affecting these molecular motors or their associated transport machinery can disrupt neuronal development, intracellular trafficking, synaptic function, and neuronal survival, thereby contributing to a broad spectrum of neurological diseases (Hirokawa et al., 2010).

Dysfunction of kinesin motors has been associated with both neurodevelopmental and neurodegenerative disorders. Different members of the

kinesin superfamily transport specific neuronal cargoes and participate in distinct processes required for neuronal organization and function. Pathogenic alterations affecting kinesin-dependent transport can disrupt the distribution of synaptic vesicle precursors, mitochondria, receptors, and other essential cargoes. Mutations in specific kinesin genes have been associated with neurological phenotypes including hereditary spastic paraplegia, peripheral neuropathies, and developmental abnormalities of the nervous system. These findings demonstrate that disturbances in kinesin-mediated transport can affect both the development and long-term maintenance of neuronal function (Hirokawa et al., 2010).

Cytoplasmic dynein is essential for retrograde transport from distal regions of neurons toward the cell body. This transport pathway supports the movement of signaling endosomes and other cellular components required for neuronal homeostasis and communication. Associated proteins and complexes, particularly dynactin, regulate dynein function and contribute to motor activity, cargo interactions, and transport efficiency. Mutations or functional abnormalities in cytoplasmic dynein and its associated transport machinery can impair retrograde axonal transport and have been linked to motor neuron diseases, peripheral neuropathies, and other neurological disorders. Thus, defects in dynein-dependent transport can compromise neuronal function by disrupting long-distance intracellular communication and cargo distribution (Hirokawa et al., 2010; De Vos and Hafezparast, 2017).

Myosin motors contribute primarily to cargo transport, localization, and organization within actin-rich neuronal compartments, including presynaptic terminals and postsynaptic regions. Through their roles in membrane trafficking, receptor localization, synaptic organization, and neuronal signaling, myosins contribute to the maintenance of synaptic function and plasticity. Abnormalities affecting myosin-dependent processes may therefore disrupt specialized neuronal activities and contribute to neurological dysfunction. These findings illustrate that motor protein-associated neurological disorders are not restricted to abnormalities of long-distance microtubule-dependent transport but may also result from disruption of localized actin-dependent trafficking and cargo positioning (Hirokawa et al., 2010).

The association between molecular motor dysfunction and neurological disease is particularly evident in disorders characterized by defective axonal transport. Because neurons depend on continuous bidirectional transport to maintain their structural and functional integrity, abnormalities in motor activity, cargo recognition, or transport regulation can have progressive consequences. Defective transport may result in abnormal cargo accumulation, impaired organelle distribution, disruption of neuronal signaling, and ultimately neuronal

dysfunction or degeneration. Motor protein and axonal transport abnormalities have therefore been implicated in several neurological and neurodegenerative diseases, demonstrating that disrupted intracellular transport is an important mechanism linking molecular motor dysfunction to disease pathogenesis (De Vos and Hafezparast, 2017).

### 2.3. Hearing Loss

Hearing loss is an important clinical manifestation of motor protein dysfunction, particularly abnormalities involving members of the myosin superfamily. Several myosin genes, including MYO3A, MYO6, MYO7A, MYO15A, MYH9, and MYH14, have been associated with hereditary hearing loss. Among these, unconventional myosins expressed in inner ear hair cells play essential roles in the development, organization, and maintenance of stereocilia. Stereocilia are actin-rich mechanosensory structures on the apical surface of cochlear hair cells, where they convert sound-generated mechanical stimuli into electrical signals. The F-actin core of stereocilia provides both structural support and a scaffold for proteins, including molecular motors and their cargoes. Consequently, pathogenic variants that impair myosin motor activity, cargo interactions, or localization within stereocilia can disrupt hair cell function and lead to sensorineural hearing loss (Miyoshi et al., 2024). Different myosin proteins perform specialized functions within cochlear hair cells, contributing to the genetic and phenotypic heterogeneity of myosin-associated hearing loss. MYO3A, MYO6, MYO7A, and MYO15A are particularly important for the development and maintenance of functional stereocilia through motor-dependent transport, localization, or anchoring of specific proteins along the actin-based cytoskeleton. Pathogenic variants in these genes are associated with autosomal dominant or autosomal recessive forms of sensorineural hearing loss. MYO7A variants can additionally cause Usher syndrome type 1B, which is characterized by congenital sensorineural hearing loss associated with vestibular dysfunction and progressive retinitis pigmentosa (Miyoshi et al., 2024).

MYO6 represents a well-characterized example of the relationship between motor protein dysfunction and hereditary hearing loss. Pathogenic variants in MYO6 are associated with both autosomal dominant non-syndromic hearing loss (DFNA22) and autosomal recessive non-syndromic hearing loss (DFNB37). Clinical manifestations vary by underlying variant and may include congenital hearing impairment or later-onset progressive sensorineural hearing loss. In a large-scale genetic analysis of Japanese families, researchers identified MYO6 variants as an important cause of autosomal dominant sensorineural hearing loss, with many affected individuals exhibiting juvenile-onset progressive hearing

impairment and marked deterioration after age 40. Experimental analyses further showed that mutant MYO6 proteins impaired the formation of actin-based cellular protrusions, supporting an association between MYO6 dysfunction, stereocilia abnormalities, and progressive hearing loss (Oka et al., 2020).

Other unconventional myosins contribute to distinct aspects of stereocilia development, organization, and function. MYO3A and MYO15A perform distinct motor-dependent functions involved in the development and maintenance of stereocilia architecture, including the localization and transport of specific proteins to stereociliary tips. MYO7A contributes to the organization of stereocilia and the function of protein complexes associated with mechanotransduction. Disruption of these specialized functions can impair the structural integrity and mechanosensory capacity of cochlear hair cells. Therefore, the clinical consequences of myosin dysfunction depend on the specific motor protein affected, its interacting partners, and its functional role within the highly organized architecture of stereocilia (Miyoshi et al., 2024). The association between myosin dysfunction and hearing loss demonstrates the importance of molecular motors beyond conventional intracellular transport. Within cochlear hair cells, myosins contribute to the transport, localization, and anchoring of proteins required for stereocilia development, maintenance, and mechanotransduction. Pathogenic variants affecting these motor-dependent processes can compromise stereocilia architecture and hair cell function, ultimately resulting in hereditary hearing loss. Thus, myosin-associated hearing disorders provide a clinically important example of how defects in actin-based molecular motors and their cargo interactions can directly contribute to human disease (Miyoshi et al., 2024).

## 3. Conclusion

Motor proteins, such as dynein, kinesin, and myosin, support intracellular transport, cytoskeletal architecture, cell division, organelle placement, and tissue-specific mechanical processes. Defects in these proteins' structure, regulation, or cargo attachments can disrupt basic cellular functions and cause a variety of human disorders because they operate at the interface between molecular motors and the cytoskeleton.

Because neurons rely heavily on long-distance axonal transport, dynein and kinesin dysfunctions are especially linked to anomalies in intracellular trafficking and neuronal function. Therefore, problems in ciliary function, neurodevelopmental disorders, neurodegenerative diseases, and peripheral neuropathies may be caused by mutations that disrupt these motors or the proteins that are connected with them. Actin-based motor systems play a variety of

physiological activities, as evidenced by the fact that myosin-associated disorders often involve problems in muscle contraction, cellular motility, hearing, and other tissue-specific functions.

In general, disorders linked to motor proteins demonstrate the intimate connection between intracellular transport, cytoskeletal movements, and cellular homeostasis. Despite tremendous progress in identifying disease-causing mutations and understanding their molecular underpinnings, many aspects of motor protein regulation and disease progression remain unclear. Further research on cytoskeletal control, motor-cargo interactions, and cellular responses to impaired transport may uncover novel therapeutic targets and provide crucial insights into disease mechanisms. Therefore, developing more accurate diagnostic and treatment approaches for illnesses caused by dynein, kinesin, and myosin malfunction will require a deeper understanding of these molecular motors.

**Conflict of Interest Statement:** The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**AI disclosure:** During the preparation of this work, the author used generative AI and AI-assisted technologies solely to improve the language, readability, and grammatical accuracy of the text. After using this service, the authors reviewed and edited the content as needed and take full responsibility for the scientific integrity and accuracy of the final published chapter.

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# Chapter 4

## Primary Ciliopathies: Histopathological Features and Cytoskeletal Basis of Human Disease

**Oya Korkmaz**[1*]

**Abstract**

Primary cilia are specialised organelles composed of microtubules that serve as crucial sensory and signalling centers in the majority of vertebrate cells. They govern embryonic development, tissue homeostasis, cellular proliferation, differentiation, and mechanotransduction through coordinated cytoskeletal organisation and intraflagellar transport (IFT). The interruption of these mechanisms leads to a varied array of hereditary conditions referred to as primary ciliopathies. Despite their genetic diversity, these disorders exhibit shared pathogenic processes characterised by faulty ciliogenesis, compromised intracellular transport, and aberrant signalling pathways, notably those involving Hedgehog, Wnt, and platelet-derived growth factor (PDGF). These molecular irregularities cause distinctive histopathological changes across many organs, including the kidneys, central nervous system, retina, liver, and skeletal system. Disorders exemplifying the multisystem ramifications of primary ciliary failure include polycystic kidney disease, Bardet–Biedl syndrome, Joubert syndrome, Meckel syndrome, and retinal ciliopathies. This chapter provides a comprehensive summary of primary cilium architecture, cytoskeletal organisation, cilium-mediated signalling pathways, histopathological changes, notable human ciliopathies, and emerging therapeutic strategies, emphasising the link between ciliary impairment and human disease.

**Keywords:** Primary cilia, Ciliopathies, Intraflagellar transport, Histopathology, Cytoskeleton

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## 1. Introduction

Primary cilia are solitary, microtubule-based organelles that project from the surface of most vertebrate cells and function as specialized sensory and signaling hubs. Unlike motile cilia with a 9+2 axonemal organization, primary cilia typically exhibit a 9+0 architecture and integrate extracellular mechanical and chemical signals to regulate cellular responses (Hilgendorf et al., 2024; Mill et al., 2023).

Primary cilia arise from the mother centriole, which forms the basal body during ciliogenesis. Their assembly and maintenance depend on coordinated cytoskeletal organization and bidirectional intraflagellar transport (IFT).

As signaling platforms, primary cilia regulate key pathways, including Hedgehog, Wnt, and platelet-derived growth factor (PDGF) signaling, thereby contributing to embryonic development, tissue homeostasis, proliferation, differentiation, and mechanotransduction (Hilgendorf et al., 2024; Lacey & Pigino, 2025; Reddy Palicharla & Mukhopadhyay, 2024).

Defects in ciliogenesis, ciliary transport, or signaling cause a heterogeneous group of inherited disorders known as ciliopathies. Despite their genetic diversity, ciliopathies share mechanisms involving impaired intracellular trafficking, disrupted signaling, and altered cytoskeletal organization (Hilgendorf et al., 2024; Moran et al., 2024; Reiter & Leroux, 2017). Because primary cilia are widely distributed across differentiated tissues, these disorders frequently affect multiple organs, particularly the kidneys, retina, central nervous system, liver, and skeleton (Hilgendorf et al., 2024; Moran et al., 2024).

Advances in molecular genetics, imaging, and experimental models have substantially improved our understanding of ciliary biology and its therapeutic implications. This chapter reviews primary cilium architecture and cytoskeletal organization, cilia-dependent signaling, histopathological alterations, major ciliopathies, and emerging therapeutic strategies.

## 2. Primary Cilium: Structure and Functional Organization

The primary cilium is a membrane-enclosed organelle that extends from the surface of most differentiated vertebrate cells, serving as a specialised site for detecting extracellular signals and facilitating intracellular communication. Its well-preserved architecture comprises four interlinked domains: the basal body, transition zone, axoneme, and ciliary membrane (Pazour, 2024) (Figure 1).

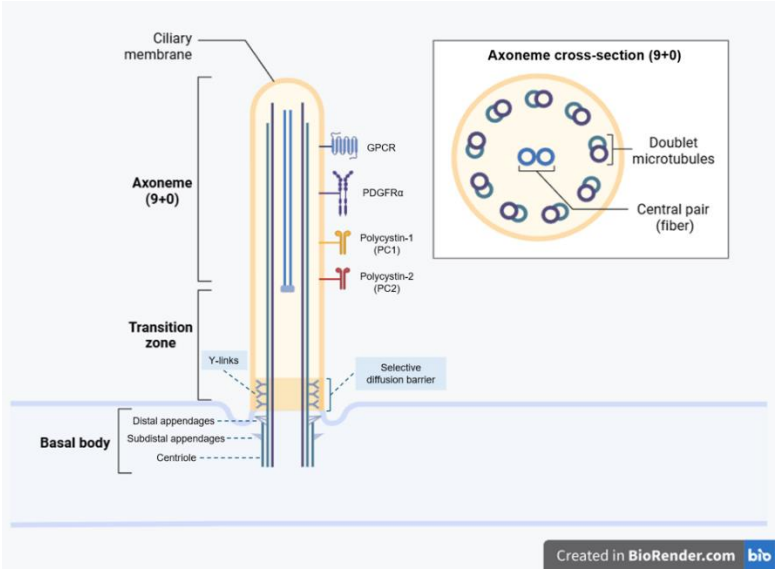



**Figure 1.** Structural organization of the primary cilium. Major structural domains of the primary cilium, including the basal body, transition zone, axoneme (9+0 microtubule arrangement), and ciliary membrane with representative membrane-associated receptors. Created with BioRender.com.

The basal body, derived from the maternal centriole, anchors the cilium to the plasma membrane, initiates ciliogenesis, and coordinates axoneme assembly. Located above the basal body, the transition zone acts as a selective diffusion barrier that controls protein transport and maintains the ciliary compartment's distinct molecular makeup (Hilgendorf et al., 2024; Mercey et al., 2024).

The axoneme, consisting of nine outer microtubule doublets organised in a distinctive 9+0 arrangement, constitutes the structural foundation of the primary cilium. Unlike motile cilia, it lacks a central pair of microtubules and is primarily involved in sensory signalling rather than movement (Hilgendorf et al., 2024). The adjacent ciliary membrane encompasses receptors, ion channels, and signalling molecules that govern developmental and homeostatic functions.

Ciliogenesis is intricately associated with the cell cycle. After cell-cycle exit, the maternal centriole transforms into the basal body, attaches to the plasma membrane, and begins axoneme formation. Ciliary development and maintenance rely on IFT, which supplies structural and signalling proteins to the developing cilium, whereas ciliary disassembly before mitosis enables the centrosome to engage in spindle formation during cell division (Hilgendorf et al., 2024; Lacey & Pigino, 2025).

### 3. Cytoskeletal Basis of Primary Cilia: Microtubules and Intraflagellar Transport

The cytoskeleton offers the essential structural framework necessary for the assembly, maintenance, and signalling of primary cilia. Microtubules form the fundamental framework of the ciliary axoneme, providing structural integrity and serving as polarised pathways for intracellular transport and mechanosensing (Hilgendorf et al., 2024; Ran & Zhou, 2025). Post-translational changes govern tubulin stability and function, affecting motor protein performance and signal transduction (Bak et al., 2024).

Because the primary cilium lacks ribosomes, ciliary proteins are synthesized in the cytoplasm and transported into the cilium through IFT, an evolutionarily conserved system essential for ciliogenesis and ciliary homeostasis. Anterograde transport, mediated by kinesin-2 and the IFT-B complex, delivers structural proteins and membrane receptors to the ciliary tip, whereas retrograde transport, driven by dynein-2 and the IFT-A complex, returns recycled proteins to the cell body (Lacey & Pigino, 2025) (Figure 2). Coordinated bidirectional transport is therefore critical for maintaining ciliary structure and signaling.

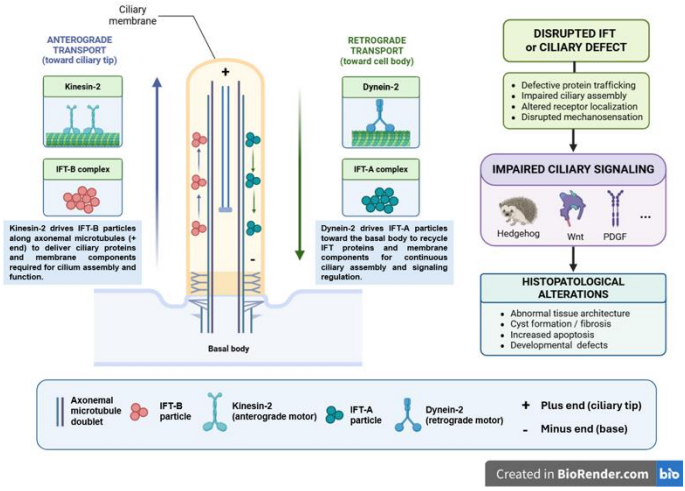



**Figure 2.** Cytoskeletal organization and intraflagellar transport in the primary cilium. The overview of anterograde (kinesin-2/IFT-B) and retrograde (dynein-2/IFT-A) intraflagellar transport and their role in maintaining ciliary signaling and structural integrity. Created with BioRender.com.

In addition to protein trafficking, IFT regulates the localization of signaling receptors involved in pathways such as Hedgehog, Wnt, and PDGF, thereby linking cytoskeletal organization to cellular communication (Hilgendorf et al., 2024). Normal ciliary function also depends on interactions between microtubules, the actin

cytoskeleton, and centrosomal proteins, which coordinate basal body positioning and ciliogenesis. Consequently, defects in axonemal microtubules or the IFT machinery disrupt signaling, impair tissue morphogenesis, and constitute a common pathogenic mechanism underlying primary ciliopathies.

**4. Ciliary Signaling Pathways**

The primary cilium functions as a specialized signaling platform by concentrating receptors and signaling molecules within a confined membrane domain. It integrates extracellular signals with intracellular networks controlling development, tissue homeostasis, proliferation, differentiation, and mechanosensation. Among cilium-associated pathways, Hedgehog, Wnt, and PDGF signaling are particularly well characterized and relevant to the pathogenesis of ciliopathies (Long et al., 2024). Hedgehog signaling is highly dependent on an intact primary cilium, where coordinated trafficking of pathway components regulates GLI transcription factor activity (Hilgendorf et al., 2024). This pathway is essential for embryonic patterning, neural development, and organogenesis, and its disruption contributes to developmental abnormalities in ciliopathies such as Joubert and Meckel syndromes (Hilgendorf et al., 2024; Reddy Palicharla & Mukhopadhyay, 2024; Zhuang, 2025).

Primary cilia also modulate Wnt signaling, which regulates cell proliferation, polarity, differentiation, and tissue organization. Ciliary proteins influence the balance between canonical and non-canonical Wnt pathways, and their dysfunction has been associated with developmental abnormalities in the kidney, nervous system, and skeleton (Coschiera et al., 2024; Niehrs et al., 2025; Vuong & Mlodzik, 2023).

Platelet-derived growth factor receptor-α (PDGFRα) localizes to the primary cilium in several cell types and regulates migration, proliferation, and tissue repair. Disrupted ciliary PDGFRα signaling may contribute to impaired wound healing, abnormal extracellular matrix remodeling, and fibrosis (Hilgendorf et al., 2024; Schneider et al., 2010). Beyond these major pathways, primary cilia interact with TGF-β, mTOR, Notch, Hippo, and calcium-dependent signaling, forming an interconnected regulatory network. Consequently, defects in ciliary architecture or intraflagellar transport can simultaneously disrupt multiple pathways, resulting in altered cell polarity, proliferation, differentiation, and extracellular matrix remodeling that contribute to the histopathological and multisystem manifestations of ciliopathies (Pala et al., 2017; Reddy Palicharla & Mukhopadhyay, 2024).

**5. Histopathological Alterations in Primary Ciliopathies**

Primary ciliopathies are characterized by a broad spectrum of histopathological abnormalities resulting from impaired ciliary signaling, defective mechanotransduction, and disrupted cytoskeletal organization. Although these

disorders are genetically heterogeneous, they share common pathological mechanisms that affect epithelial architecture, tissue morphogenesis, and organ development. As a result, characteristic histopathological changes occur across multiple organ systems, particularly the kidneys, central nervous system, retina, liver, pancreas, and skeleton (Alzarka et al., 2025; Sekar & Sebire, 2024).

The kidney is the organ most frequently affected in primary ciliopathies because renal tubular epithelial cells rely on primary cilia to sense tubular fluid flow and regulate intracellular signaling. Loss of ciliary function results in abnormal epithelial proliferation and cystogenesis, producing characteristic histopathological findings such as tubular dilatation, epithelial flattening, interstitial fibrosis, and progressive disruption of renal architecture (Sekar & Sebire, 2024; Wolf et al., 2024).

Primary cilia are essential for normal neurodevelopment by regulating neuronal proliferation, migration, and tissue patterning. Consequently, ciliary dysfunction is associated with cerebellar vermis hypoplasia, abnormal neuronal organization, and brainstem malformations (Montero Torres et al., 2024; Spahiu et al., 2023; Zhang et al., 2025). These developmental abnormalities largely reflect impaired Hedgehog signaling during embryogenesis.

Photoreceptor cells depend on an intact connecting cilium for continuous transport of phototransduction proteins. Defective ciliary trafficking leads to progressive degeneration of photoreceptor cells, characterized histologically by loss of photoreceptor outer segments, retinal thinning, and apoptosis, ultimately resulting in irreversible visual impairment (Gupta & Pazour, 2024; Ran et al., 2024; Xu et al., 2024).

Primary cilia also regulate epithelial homeostasis in the liver and pancreas as well as chondrocyte organization within the growth plate. Histopathological alterations include ductal plate malformation, portal fibrosis, ductal dilatation, and disorganized growth plate architecture, reflecting defective tissue morphogenesis and extracellular matrix remodeling (Alzarka et al., 2025).

Despite organ-specific differences, several pathological findings are consistently observed across primary ciliopathies. These include loss of epithelial polarity, abnormal tissue architecture, defective tubular or ductal morphogenesis, progressive fibrosis, extracellular matrix remodeling, and increased apoptosis (Corbeil et al., 2025; Sekar & Sebire, 2024). Taken together, these changes represent the tissue-level consequences of disrupted ciliary signaling and impaired intracellular transport (Table 1).

**Table 1.** Histopathological alterations in primary ciliopathies. Overview of the major organ-specific histopathological findings and their underlying mechanisms.

| Organ | Major Histopathological Findings | Underlying Mechanism |
|---|---|---|
| Kidney | Tubular dilatation, cysts, fibrosis | Impaired mechanosensation and ciliary signaling |
| Brain | Cerebellar hypoplasia, abnormal neuronal organization | Disrupted Hedgehog signaling |
| Retina | Photoreceptor degeneration, retinal thinning | Defective intraflagellar transport |
| Liver | Ductal plate malformation, portal fibrosis | Abnormal cholangiocyte signaling |
| Skeleton | Growth plate disorganization | Altered Hedgehog/Wnt signaling |

Histopathological evaluation therefore links molecular defects to clinical manifestations, facilitating both disease classification and genotype–phenotype correlation. Advances in digital pathology and molecular imaging are expected to further improve the understanding and diagnosis of primary ciliopathies.

## 6. Major Human Primary Ciliopathies

Primary ciliopathies comprise a diverse group of inherited disorders caused by mutations affecting proteins involved in ciliogenesis, intraflagellar transport, transition zone integrity, and ciliary signaling. Despite marked genetic heterogeneity, these disorders share common pathogenic mechanisms involving defective intracellular trafficking, disrupted developmental signaling, and impaired tissue morphogenesis. The kidneys, central nervous system, retina, liver, and skeleton are the organs most frequently affected (Alzarka et al., 2025; Hilgendorf et al., 2024) (Table 2).

**Table 2.** Representative primary ciliopathies and their key characteristics. Summary of the major primary ciliopathies, associated genes, principal organs affected, and key clinical features.

| Disorder | Major Gene(s) | Main Organ(s) | Key Feature |
|---|---|---|---|
| Polycystic kidney disease | PKD1, PKD2 | Kidney | Renal cyst formation |
| Bardet–Biedl syndrome | BBS genes | Retina, kidney | Multisystem ciliopathy |
| Joubert syndrome | AHI1, CEP290, TMEM67 | Brain | Cerebellar malformation |
| Meckel syndrome | TMEM67, MKS1, CEP290 | Brain, kidney, liver | Severe developmental defects |
| Retinal ciliopathies | CEP290, RPGR | Retina | Progressive retinal degeneration |

Polycystic kidney disease (PKD) is the most extensively studied ciliopathy and results primarily from mutations in PKD1 or PKD2, which encode the mechanosensory proteins polycystin-1 and polycystin-2 (Esarte Palomero et al., 2023). Loss of ciliary mechanosensation disrupts calcium-dependent signaling and promotes abnormal epithelial proliferation, leading to progressive renal cyst formation and chronic kidney disease (Alshriem et al., 2025).

Bardet–Biedl syndrome is an autosomal recessive ciliopathy caused by mutations in genes encoding components of the BBSome, a protein complex required for ciliary membrane trafficking. Defective receptor transport results in a multisystem disorder characterized by retinal degeneration, obesity, renal abnormalities, postaxial polydactyly, cognitive impairment, and hypogonadism (Dollfus et al., 2024; Shoemaker, 2024).

Joubert syndrome is primarily associated with mutations affecting transition zone proteins that regulate ciliary signaling during embryonic development. The disorder is characterized by cerebellar malformations, developmental delay, hypotonia, abnormal eye movements, and the characteristic molar tooth sign on neuroimaging, reflecting abnormal hindbrain development (Montero Torres et al., 2024; Spahiu et al., 2023).

Meckel syndrome is a severe developmental ciliopathy caused by mutations affecting proteins involved in ciliogenesis and transition zone organization. It presents with occipital encephalocele, cystic kidneys, congenital hepatic fibrosis, and postaxial polydactyly and is typically lethal during the prenatal or neonatal period because of profound developmental abnormalities (Hartill et al., 2017).

Several inherited retinal disorders result from mutations affecting proteins required for ciliary transport within photoreceptor cells. Defective trafficking of phototransduction proteins causes progressive photoreceptor degeneration, leading to irreversible vision loss (Gupta & Pazour, 2024; Ran et al., 2024; Xu et al., 2024). Retinal degeneration may occur in isolation or as part of multisystem ciliopathies such as Bardet–Biedl and Joubert syndromes (Dollfus et al., 2024; Spahiu et al., 2023).

Although primary ciliopathies differ in their genetic causes and clinical presentation, they share several fundamental mechanisms, including impaired ciliogenesis, defective intraflagellar transport, disrupted signaling, and abnormal mechanosensation. These common molecular defects explain the overlapping clinical manifestations across disorders and provide a basis for developing targeted therapeutic strategies.

## 7. Emerging Therapeutic Approaches

Current management of primary ciliopathies remains largely supportive, focusing on organ-specific complications. However, advances in molecular genetics and ciliary biology have shifted research toward therapies targeting the underlying mechanisms of ciliary dysfunction. Gene-based therapies, including viral vector-mediated gene replacement, CRISPR/Cas9 genome editing, and antisense oligonucleotides, offer promising strategies to correct disease-causing genetic defects, though most remain preclinical or in early clinical development (McDonald & Wijnholds, 2024). Another major approach involves the pharmacological modulation of ciliary signaling pathways. Small-molecule compounds targeting mTOR, Hedgehog, Wnt, and cAMP signaling have shown encouraging results in experimental models by improving signaling homeostasis and reducing disease progression (Hilgendorf et al., 2024; Liu et al., 2024). In addition, patient-derived induced pluripotent stem cells (iPSCs) and three-dimensional organoid models provide valuable platforms for investigating disease mechanisms and evaluating candidate therapies, supporting the development of personalized treatment strategies (Kuzinska et al., 2024). Although curative therapies are not yet available, continued advances in molecular genetics, disease modeling, and targeted therapeutics are expected to accelerate the translation of precision medicine into the clinical management of primary ciliopathies.

## 8. Conclusions

Primary cilia are highly specialized sensory organelles that play central roles in cellular signaling, tissue homeostasis, and vertebrate development. Their structural integrity depends on coordinated cytoskeletal organization and efficient intraflagellar transport, which together regulate multiple signaling pathways essential for normal organ development and function. Disruption of ciliary architecture or signaling results in impaired intracellular communication, abnormal tissue morphogenesis, and the characteristic histopathological alterations observed in primary ciliopathies. Although these disorders are genetically diverse, they share common molecular mechanisms involving defective ciliogenesis, impaired protein trafficking, and dysregulated signaling, leading to multisystem disease affecting the kidneys, central nervous system, retina, liver, and skeleton. Advances in molecular genetics, disease modeling, and precision medicine have substantially improved our understanding of ciliopathy pathogenesis and are driving the development of targeted therapeutic strategies. Continued integration of molecular, histopathological, and translational research will be essential to improve diagnosis and advance effective treatments for patients with primary ciliopathies.

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**AI disclosure:** During preparation of this book chapter, the author used AI-assisted tools (DeepL, Grammarly, and Grok) for language editing and to improve the text's clarity and readability.

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# Chapter 5

## Nuclear Cytoskeleton and Nuclear Envelope Disorders: Histopathology of Laminopathies

**Aysegul Burcin Yildirim**[1*]

**Abstract**

The nuclear lamina is a dense, fibrous structure located just beneath the inner nuclear membrane in the nucleus of eukaryotic cells. This network consists of lamin proteins, which possess intermediate filament characteristics, and various proteins bound to the nuclear membrane. In mammalian cells, lamin proteins are generally divided into two main groups: lamin A/C and lamin B. The nuclear lamina not only provides mechanical strength to the nucleus but also serves as a key component of the LINC complex, which connects the nucleus to the cytoplasmic cytoskeleton. This complex contains SUN proteins on the inner nuclear membrane and nesprins, also known as KASH proteins, on the outer nuclear membrane. This allows mechanical forces originating from the cytoskeleton to be transmitted to the nucleus and establishes a physical connection between the nucleus and other parts of the cell. Abnormalities in the structure or function of lamin proteins can cause various inherited diseases known as laminopathies. Emery-Dreifuss muscular dystrophy and Hutchinson-Gilford progeria syndrome are among the best-known examples of this group of diseases. Histopathological examination in laminopathies includes adipocyte morphometry, fibrosis staining, immunohistochemical detection of inflammatory cells, and electron microscopic examination. These techniques are primarily supportive; the definitive diagnosis of laminopathies should be based on clinical findings, family history, and molecular genetic analysis. This chapter examines the architecture of the nuclear lamina, the interactions of lamin proteins with other cellular components, and the role of this dynamic structure in cellular functions and disease development.

**Keywords:** Laminopathies, Lipodystrophy, Hutchinson-Gilford progeria syndrome (HGPS)

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## Introduction

### 1. Nuclear Lamina

The nuclear lamina, located on the side of the nuclear membrane facing the nucleoplasm, is a filamentous protein network with a thickness ranging from 30 to 100 nanometers. The primary components of this network are lamin proteins belonging to the type V intermediate filament class (Sobo et al., 2024). Beyond protecting the nucleus from mechanical stress, this structure regulates cellular mechanisms such as gene expression, DNA repair and replication, and chromatin architecture (Tenga & Medalia, 2020; Vahabikashi et al., 2022). Architectural integrity is not maintained by lamin alone; inner membrane proteins such as emerin, LBR (Lamin B Receptor), LAP2 (Lamina-Associated Polypeptide 2), and SUN (Sad1/UNC-84), as well as transcription factors and chromatin-regulating molecules, are also part of the network. Through its direct structural connection with chromatin, this network localizes heterochromatin to the nuclear periphery, thereby governing the genome's spatial organization (Wilson & Foisner, 2010).

### 2. Lamin A/C and B Proteins

#### 2.1. Classification and Genetic Origin

In mammals, lamin proteins are categorized into two major classes, all of which are transcribed from just three genes: LMNA (Lamin A), LMNB1 (Lamin B1) and LMNB2 (Lamin B2) (Burke & Stewart, 2013):

**Type A lamin:** The *LMNA* gene produces the isoforms lamin A, lamin C, lamin AΔ10, and lamin C2 through alternative splicing. Lamin A and lamin C are the major isoforms in somatic cells. Lamin A is first synthesized as prelamin A and is converted to mature lamin A following post-translational modifications. Type A lamin proteins are highly expressed in differentiated cells; their expression is low in stem cells (Swift et al., 2013). LMNA mutations cause at least nine different laminopathies, such as progeria, muscular dystrophy, and lipodystrophy (Stewart et al., 2007; Worman, 2012).

**Type B lamin proteins:** *LMNB1* encodes lamin B1, while *LMNB2* encodes lamin B2 and B3. Type B lamin proteins are expressed in nearly all nucleated cells (Rusiñol & Sinensky, 2006). Deleting Lmnb1 or Lmnb2 in mice is lethal shortly after birth, with the most pronounced abnormalities in brain development (Odell & Lammerding, 2023). Type B lamin proteins remain associated with the nuclear envelope during mitosis and maintain nuclear integrity (Odell & Lammerding, 2023; Peter & Stick, 2012).

### 3. LINC Complex

Acting as a structural bridge, the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex binds the nucin shortlear components to the cytoskeletal network. It consists of SUN (Sad1/UNC-84) domain proteins in the inner nuclear membrane and KASH (Klarsicht/ANC-1/Syne/UNC-84 homology) domain proteins in the outer nuclear membrane, such as nesprins (Crisp et al., 2006).

- **SUN proteins (SUN1, SUN2):** Interact with lamin proteins on the nucleoplasmic side and bind to KASH proteins in the perinuclear region.
- **KASH proteins (nesprin-1, nesprin-2, nesprin-3, nesprin-4):** On the cytoplasmic side, they bind to the actin cytoskeleton, intermediate filaments (via plectin), or microtubules.

**Through the LINC complex:** Extracellular mechanical stimuli are transmitted to the nucleus via the cytoskeleton; the nucleus is positioned correctly within the cell; the size and shape of the nucleus are regulated; and chromatin organization is influenced by mechanical signals (Luxton et al., 2010).

#### 3.1. Disease Associations

Mutations in LINC complex components lead to a wide range of diseases.

- **Muscle disorders**: Nesprin and SUN mutations are associated with muscular dystrophy and dilated cardiomyopathy (Sosa et al., 2013; Stroud, 2018).
- **Neurological disorders**: LINC complex mutations are observed in neurodegenerative and psychiatric disorders (Kuwako & Suzuki, 2024; Sosa et al., 2013).
- **Premature aging**: Dysregulation of the complex disrupts nuclear integrity in premature aging phenotypes such as Hutchinson-Gilford progeria syndrome (HGPS) (Gurusaran & Davies, 2021; Lansweers et al., 2025).
- **Vascular pathologies**: LINC mutations contribute to atherosclerosis by impairing endothelial function and vascular remodeling (Ferreira et al., 2026).

### 4. Laminopathies

Laminopathies are a group of genetic diseases with diverse clinical presentations that result from mutations in genes encoding A-type and B-type lamins, the major structural proteins of the nuclear envelope (Leeuw et al., 2018; Worman, 2012). Most cases result from point mutations in the LMNA gene, which encodes lamin A and C proteins expressed in nearly all differentiated cells of the body (Donnaloja et al., 2020; Leeuw et al., 2018). Although lamin deficiency disrupts nuclear architecture and chromatin organization, the damage

caused by these mutations is generally limited to specific tissues. This tissue specificity shows that lamin proteins are not merely structural scaffolds but also perform active regulatory functions (Zhao et al., 2026).

**Clinical Spectrum and Classification:** Mutations in the LMNA gene cause at least 15 rare diseases. These diseases are grouped into three main categories based on the affected tissue (Leeuw et al., 2018; Vahabikashi et al., 2022). Inheritance is predominantly autosomal dominant, although autosomal recessive forms also exist (Donnaloja et al., 2020). The mutation's location within the gene directly determines the disease's clinical presentation. For example, mutations in the Ig-fold region cause lipodystrophy, while defects in the C2 and C1b regions cause myopathies (Donnaloja et al., 2020). The three main clinical groups of laminopathies and the primary tissues affected are summarized in Table 1.

**Table 1.** The three clinical groups of laminopathies and the primary tissues affected.

| **Disease Group** | | **Key Examples** | **Affected Tissue/System** |
|---|---|---|---|
| **Muscular** | | Emery-Dreifuss muscular dystrophy, Dilated cardiomyopathy | Skeletal and cardiac muscle (Vahabikashi et al., 2022; Worman, 2012) |
| **Metabolic** | | Familial Partial Lipodystrophy (FPLD) | Adipose tissue (Donnaloja et al., 2020; Vahabikashi et al., 2022) |
| **Progeroid** | | Hutchinson-Gilford Progeria Syndrome (HGPS) | Multisystem involvement, accelerated aging (Donnaloja et al., 2020; Vahabikashi et al., 2022) |

### 4.1. Emery-Dreifuss Muscular Dystrophy (EDMD)

Emery-Dreifuss muscular dystrophy is an inherited muscle disorder characterized by early contractures, slowly progressive humeroperoneal muscle weakness, and cardiac conduction abnormalities (Heller et al., 2020; Meinke, 2018). The most critical concern is that cardiac involvement increases the risk of arrhythmia, cardiomyopathy, and sudden death (Granata et al., 2026; Madej-Pilarczyk, 2018). EDMD is genetically heterogeneous and includes X-linked, autosomal dominant, and, more rarely, autosomal recessive forms (Granata et al., 2026; Meinke, 2018). The most commonly associated genes are EMD and LMNA, although Nesprin-1, Nesprin-2, SUN1, SUN2, and TTN (Titin) have also been reported (Anandan et al., 2025; Meinke, 2018). Muscle symptoms often begin in childhood and generally progress slowly (Madej-Pilarczyk, 2018). In

contrast, cardiac disease is the most significant factor determining prognosis; therefore, genetic confirmation and regular cardiac monitoring are paramount for diagnosis (Granata et al., 2026; Heller et al., 2020). In short, Emery-Dreifuss muscular dystrophy is a rare but clinically significant group of muscular dystrophies associated with nuclear envelope proteins that affect the muscles and, in particular, the heart.

### 4.2. Dilated Cardiomyopathy (DCM)

Dilated cardiomyopathy (DCM), defined by increased ventricular diameter and loss of systolic function, is a heart muscle pathology not of ischemic origin (Heymans et al., 2023). With a prevalence of approximately 1 in 250, this clinical condition is the most common cause of non-ischemic heart failure. It is also one of the leading causes of sudden cardiac death and heart transplant indications in young patients (Ferreira et al., 2023; Gigli et al., 2025; Japp et al., 2016). Symptoms typically begin in patients in their 30s and 40s; the incidence in men is three times higher than in women.

Genetic mutations or environmental stressors affecting the heart muscle ultimately result in the same cellular response, namely DCM (Japp et al., 2016). The etiological classification consists of two main categories: genetic and acquired (Heymans et al., 2023). Viral myocarditis, endocrine disorders, autoimmune processes, pregnancy, toxins, and chemotherapy agents constitute the acquired category. However, the detection of pathogenic mutations in 5 to 15 percent of patients classified as having acquired forms confirms that the pathogenesis of the disease involves a complex interaction between genetic predisposition and environmental factors (Gigli et al., 2025; Heymans et al., 2023). Genetic causes account for approximately 30–50% of cases and result from mutations in genes encoding cytoskeletal, sarcomere, desmosome, and nuclear envelope proteins. Familial DCM is defined by the presence of two or more cases of DCM in a family or a history of sudden death before the age of 35. The most common mutations are found in the TTN, LMNA, and MYH7(Myosin Heavy Chain 7) genes (Eldemire et al., 2024; McNally & Mestroni, 2017; Wang et al., 2025). A comprehensive gene curation has confirmed that 12 out of 51 genes (TTN, LMNA, MYH7 etc.) are definitively associated with DCM (Jordan et al., 2021). Patients most commonly present with symptoms of congestive heart failure, but arrhythmia, syncope, thromboembolism, or sudden death may also be the initial presentation (Ferreira et al., 2023; Weintraub et al., 2017). The disease has a long asymptomatic preclinical phase, and subclinical DCM may be diagnosed during family screenings. Echocardiography and Cardiovascular Magnetic Resonance are the primary diagnostic tools for identifying myocardial

fibrosis and ventricular dysfunction (Ferreira et al., 2023; Japp et al., 2016). The presence of myocardial fibrosis is a critical prognostic marker for predicting the risk of sudden cardiac death and the likelihood of left ventricular functional recovery (Japp et al., 2016).

### 4.3.Lipodystrophy

Lipodystrophy is a heterogeneous group of disorders characterized by partial or widespread loss of adipose tissue and abnormal fat distribution in the body. It can be genetic or acquired; phenotypically, it is classified into generalized and partial forms. Adipose tissue loss is associated with metabolic disorders such as insulin resistance, hypertriglyceridemia, hepatic steatosis, and metabolic syndrome. Inherited lipodystrophies: These are associated with genetic mutations; examples include Hutchinson–Gilford progeria syndrome (HGPS), mandibuloacral dysplasia (MAD), and familial partial lipodystrophy (FPLD), all of which fall under the category of laminopathies (Krüger et al., 2024).

#### 4.3.1. Hutchinson-Gilford Progeria Syndrome (HGPS)

A single point mutation in the *LMNA* gene disrupts normal lamin A production and leads to the formation of a defective protein called progerin. HGPS is characterized by developmental delay in early infancy, alopecia, sclerodermatoid skin hardening, lipodystrophy, and severe atherosclerosis (Hennekam, 2006). Vascular involvement is the primary cause of fatal outcomes (Olive et al., 2010). Vascular smooth muscle cells (VSMCs) in the aortic media are depleted and replaced by collagen fibers (Hamczyk & Andrés, 2019). Atherosclerotic lesions are fibro-calcific in nature (Olive et al., 2010). The adventitia becomes markedly thickened and fibrotic. Progerin accumulates primarily in vascular endothelial and smooth muscle cells. It has also been reported that the amount of progerin in coronary arteries increases with age during physiological aging (McClintock et al., 2006; Olive et al., 2010).

#### 4.3.2. Familial partial lipodystrophy (FPLD)

Progressive depletion of adipose tissue is the fundamental feature of familial partial lipodystrophy (FPLD), an uncommon genetic condition. The strong correlation between this disease and metabolic syndrome has recently positioned FPLD at the center of related medical research. The disorder is currently divided into six distinct subtypes based on their genetic origins. While FPLD Type 1 has a polygenic etiology, the other variants emerge from specific mutations in genes such as LMNA, PPARG (Peroxisome Proliferator-Activated Receptor Gamma), PLIN1(Perilipin 1), CIDEC (Cell Death-Inducing DFFA-Like Effector C) and

LIPE (Lipase E). Among these, FPLD Type 2 stands out, as it is driven by *LMNA* defects that frequently trigger premature aging in patients (Hussain & Garg, 2016; Krüger et al., 2024).

## 5. Diagnostic Approaches

### 5.1. Histopathological Findings and Diagnostic Methods

Histopathological examination in laminopathies includes adipocyte morphometry, fibrosis staining, immunohistochemical detection of inflammatory cells, and electron microscopy. In LMNA-associated disorders, evaluating fibroblast nuclear morphology using lamin A/C immunofluorescence is a complementary method that supports the pathogenicity of ambiguous LMNA variants (Krüger et al., 2024; van Tienen et al., 2019). These techniques are primarily supportive; the definitive diagnosis of laminopathies should be based on clinical findings, family history, and molecular genetic analysis.

## 6. Future Therapeutic Strategies

Treatment for laminopathies is aimed more at reducing pathological protein accumulation and metabolic complications than at completely eliminating the genetic cause of the disease. In HGPS, improvements in certain clinical findings such as body weight, arterial stiffness, bone structure, and hearing have been reported with lonafarnib, a farnesyltransferase inhibitor that reduces progerin farnesylation; however, a significant portion of the evidence is based on non-randomized studies. Antisense or morpholino oligonucleotides aim to reduce pathological LMNA splicing and progerin production, while CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9) and other gene-editing approaches aim to correct the disease-causing mutation; these methods remain largely experimental. In lipodystrophies such as FPLD2, metreleptin may improve glycemic control and lipid metabolism, particularly in patients with low leptin levels and severe metabolic dysfunction; additionally, clinicians may administer insulin, metformin, lipid-lowering medications, and GLP-1 (Glucagon-Like Peptide-1) receptor agonists based on individual needs. Rapamycin, baricitinib, and similar mTOR (Mechanistic Target of Rapamycin) or inflammation-targeted therapies are being investigated to reduce prelamin A/progerin accumulation, cellular aging, and adipogenesis defects (Atalaia et al., 2021; Krüger et al., 2024). In LMNA-associated DCM, activation of the ERK1/2 (Extracellular Signal-Regulated Kinase 1/2) signaling pathway contributes to myocardial fibrosis. MEK1/2 (Mitogen-Activated Protein Kinase Kinase 1/2) inhibitors have shown the potential to slow the progression

of fibrosis (Muchir et al., 2012). However, the current evidence for these approaches is largely limited to cellular and preclinical studies.

## 7. Conclusion

Ultimately, gaining a comprehensive understanding of the molecular architecture, protein interactions, and biomechanics of nuclear lamina is critical to understanding the cellular damage caused by genetic abnormalities and to leading the development of innovative treatment strategies for these rare metabolic and genetic syndromes.

**Conflicts of Interest:** The authors declare no conflicts of interest.

**Declaration of AI:** The author used Google Gemini/Consensus tools during the writing process of this study to organize ideas and improve the flow of the text. After using artificial intelligence, the author reviewed and approved the entire text. The author bears full responsibility for the data, ideas, and final publication presented in this study.

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# Chapter 6

## Cytoskeletal Dysfunction in Developmental and Congenital Disorders

**Gizem Gamze Tas** [1*]

**Abstract**

Embryonic development is a dynamic process in which cellular proliferation, differentiation, migration, and reorganization occur in a coordinated manner. During this process, the ability of cells to change their shape, position, and interactions with their surrounding environment is closely associated with continuous cytoskeletal remodeling. Actin filaments, microtubules, and intermediate filaments work together in a wide range of events, from regulating cell polarity and shape to cell division and migration, as well as cell-cell interactions and tissue and organ formation. The conversion of mechanical stimuli into cellular signals and their integration with developmental signaling networks further demonstrate the functional importance of the cytoskeleton during development. This chapter examines the roles of actin filaments, microtubules, and intermediate filaments during embryonic development and their contributions to regulating cellular processes. Cytoskeletal remodeling during cell polarity, division, migration, and morphogenesis, together with interactions involving signaling pathways, mechanotransduction, primary cilia, and the extracellular matrix, is discussed. The effects of cytoskeletal disruption on neuronal, craniofacial, cardiac, musculoskeletal, and renal development, as well as its contribution to ciliopathy development, are evaluated. The final section summarizes current research approaches and future directions aimed at elucidating the molecular mechanisms underlying cytoskeleton-associated developmental disorders.

**Keywords:** Embryonic development, Cytoskeletal dysfunction, Congenital disorders, Morphogenesis, Cellular dynamics, Ciliopathies

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## 1. Introduction

The cytoskeleton comprises interconnected actin filaments, microtubules, and intermediate filaments that collectively shape cellular architecture and coordinate processes such as division, migration, intracellular transport, mechanotransduction, and signal transduction. During development, it is essential for neuronal migration, cell polarization, tissue and organ morphogenesis, and differentiation; consequently, cytoskeletal dysfunction is associated with neurodevelopmental, congenital brain, craniofacial, cardiac, and musculoskeletal disorders. The cytoskeleton also interacts closely with primary cilia, extracellular matrix, and developmental signaling pathways, and disruption of these interactions contributes to ciliopathies and other pathological processes.

This chapter reviews the molecular basis and cellular consequences of cytoskeletal dysfunction, focusing on actin, microtubules, and intermediate filaments and their interactions with signaling and mechanotransduction pathways. It discusses their roles in neuronal, craniofacial, cardiac, musculoskeletal, and renal developmental disorders, along with the molecular basis of ciliopathies and their multisystem manifestations, highlighting their relevance to disease pathogenesis and therapeutic development.

## 2. Cytoskeleton During Embryonic Development

Embryogenesis requires the precise coordination of proliferation, differentiation, cell movement, and tissue remodeling. Throughout this process, continuous reorganization of the cytoskeleton enables cells to adapt their shape, position, and mechanical properties to developmental requirements (Sonnen & Janda, 2021). The cytoskeleton, composed of microtubules, actin filaments, and intermediate filaments, plays essential roles in maintaining cellular architecture, cell movement, mechanical force transmission, intracellular transport, and signal transduction (Momotyuk et al., 2025). Throughout embryogenesis, the cytoskeleton undergoes continuous remodeling in response to developmental demands and contributes to the regulation of cell division, migration, and tissue architecture, particularly during blastocyst formation, gastrulation, neurulation, and organogenesis. Therefore, normal embryonic development depends on the coordinated function of cytoskeletal components (Clarke & Martin, 2021).

### 2.1. Cytoskeleton and Cell Polarity

Cytoskeletal remodeling contributes to this spatial organization by directing polarity proteins and intracellular cargo to specific cellular domains. Actin primarily supports cortical remodeling and adhesion, whereas microtubules provide directional routes for intracellular transport (Bera & Loeffler, 2025). In

polarized cells, the distribution of regulatory molecules is coordinated with the oriented organization of actin filaments and microtubules. Apical-basal and planar cell polarity are particularly important for organogenesis, tissue architecture, and regulating cell orientation (Tarchini & Lu, 2019; Thapa et al., 2023).

During symmetry breaking at the onset of cell polarization, the cytoskeleton regulates the transport and redistribution of polarity regulators to specific cellular regions. Actin filaments contribute to cortical remodeling, adhesion, and membrane protrusion formation, whereas microtubules mediate the directed transport of organelles, vesicles, and polarity proteins (Mastrogiovanni et al., 2020; Simoes-da-Silva & Barisic, 2025).

Rho GTPases (Cdc42, Rac1, and RhoA), the Arp2/3 complex, and formins further regulate these processes. In particular, Cdc42 plays a critical role in establishing the polarity axis (Xing et al., 2025; Zhang et al., 2021). The stabilization of microtubule plus (+) ends at the cortex, together with (+TIP) proteins such as EB1, supports microtubule orientation. Myosins, kinesins, and dyneins mediate the directed intracellular transport of organelles, vesicles, and polarity regulators (D'Souza et al., 2023).

The maintenance of cell polarity relies on the coordinated functions of the PAR (PAR3-PAR6-aPKC), Crumbs, and Scribble complexes. By interacting with actin and microtubules, these complexes help establish apical and basolateral domains. The link between cell polarity and cell fate during early embryonic development provides an important example. In the 16-cell embryo, apical F-actin organization and asymmetric inheritance of keratins in outer cells support the cortical accumulation of PAR6 and aPKC. Apical polarization promotes *Cdx2* expression and trophectoderm fate through the Hippo-YAP/TEAD4 axis, whereas cytoplasmic retention of YAP in inner cells contributes to inner cell mass fate (Lim & Plachta, 2021). Actin-microtubule interactions contribute to the maintenance of polarity and are essential for epithelial integrity, cell migration, asymmetric cell division, and organogenesis (Peng et al., 2020).

### 2.2. Cytoskeleton in Cell Division and Proliferation

Cell division and proliferation are essential for embryonic development, tissue regeneration, and growth. Actin filaments, microtubules, and intermediate filaments coordinate chromosome segregation, mitotic spindle formation, cytokinesis, and cellular integrity throughout the cell cycle (Gibieza & Petrikaite, 2021; Rizzelli et al., 2020). During mitosis, microtubules form the mitotic spindle, while kinesin and dynein regulate chromosome movement and spindle

stability. Disruption of microtubule dynamics can cause chromosome segregation errors, aneuploidy, and genomic instability (Vicente & Wordeman, 2019).

Mitotic spindle orientation is critical for determining the plane of cell division and regulating the developmental fate of daughter cells (Hadjisavva & Skourides, 2025). In stem and progenitor cells, symmetric versus asymmetric division is closely linked to cell differentiation, tissue morphogenesis, and organogenesis (Chhabra & Booth, 2021). Astral microtubules extending from centrosomes to the cell cortex help align the spindle through forces generated by cortical dynein-dynactin complexes, supported by the cortical actin network (Okumura et al., 2018). Dishevelled (Dvl), Rho GTPases, and formin-mediated pathways further coordinate actin-microtubule interactions, while cell shape and mechanical forces contribute to the positioning of the division axis (Alam et al., 2025).

During cytokinesis, actin filaments and myosin II form a contractile ring at the cell equator, generating the cleavage furrow that separates the cell into two daughter cells. RhoA GTPase-mediated actomyosin contraction is essential for the completion of cytokinesis (Carim et al., 2020; Verma et al., 2019). Collectively, these mechanisms establish the cytoskeleton as a central regulator of cell division. Dysregulation of these processes is associated with various pathological conditions, including genomic instability, developmental abnormalities, neurodevelopmental disorders, polycystic kidney disease, and cancer.

### 2.3. Cytoskeleton in Cell Migration, Morphogenesis, and Organogenesis

Cell migration is a critical biological process in embryonic development, tissue morphogenesis, and organogenesis, requiring the coordinated regulation of cell polarity, movement, and responses to environmental cues. During this process, actin filaments support lamellipodia and filopodia formation, microtubules contribute to cell polarity and vesicular transport, and intermediate filaments provide cellular and nuclear mechanical resilience (Conboy et al., 2024; Lim et al., 2026).

The Rho GTPases RhoA, Rac1, and Cdc42 coordinate actin remodeling, focal adhesion, and actomyosin contractility. Rac1 and Cdc42 promote actin polymerization at the leading edge, whereas RhoA-ROCK-mediated myosin II activation facilitates rear retraction, thereby coordinating protrusion, adhesion, and contraction (Mishra et al., 2026).

During morphogenesis and organogenesis, these mechanisms regulate not only individual cell movements but also collective cell migration and tissue architecture (Matthew et al., 2024).

Cadherin and integrin-mediated cell-cell and cell-extracellular matrix interactions establish mechanical coupling with the cytoskeleton, facilitating force transmission across tissues (Katoh, 2025). During mesenchymal condensation, an early stage of organogenesis, cell organization driven by FGF, BMP, WNT, Hedgehog, and TGF-β signaling is supported by Rac1-mediated actin polymerization and RhoA/ROCK-dependent actomyosin contractility. Contractile forces transmitted through F-actin regulate intercellular tension and contribute to shaping the developing organ primordium (He et al., 2025).

As organ development progresses, interactions between the cytoskeleton and extracellular matrix become increasingly important. Integrin-mediated linkage of fibronectin and collagen to the actin cytoskeleton supports focal adhesion formation, while changes in matrix stiffness influence cell migration, proliferation, and actin organization through integrin-FAK, MAPK, and Rho/ROCK signaling (Hassan et al., 2026; Katoh, 2025). Mechanical forces generated by actomyosin and transmitted to the nucleus through focal adhesions regulate mechanosensitive transcriptional regulators such as YAP/TAZ, β-catenin, and MRTF/SRF, thereby contributing to cell fate and differentiation (Long et al., 2025; Nam et al., 2026). Accordingly, organ formation emerges from the continuous integration of developmental signaling with forces generated and transmitted by the cytoskeletal system (He et al., 2025). Organoid studies further highlight the importance of coordinated actin dynamics, cell-matrix interactions, and mechanotransduction in organ development (Zhang et al., 2026).

## 3. Molecular Mechanisms of Cytoskeletal Dysfunction

Microtubules, actin filaments, and intermediate filaments undergo continuous remodeling, enabling cells to respond to environmental cues, change direction, divide, and differentiate. The proper execution of these processes depends on the precise synthesis, organization, and regulation of cytoskeletal components (Momotyuk et al., 2025).

### 3.1 Actin dysregulation

Actin filaments support essential cellular processes, including cell shape, mechanical integrity, adhesion, migration, cell division, and signal transduction (Gibieza & Petrikaite, 2021). The polymerization-depolymerization cycle between G-actin and F-actin is regulated by the Arp2/3 complex, formins, profilin, cofilin, and capping proteins (Bai et al., 2023) (Figure 2A). Mechanical tension generated by actin polymerization promotes nuclear translocation of the Hippo pathway effector YAP1, thereby supporting the transcription of genes

involved in proliferation, differentiation, and tissue homeostasis, whereas actin dysregulation can disrupt this process (Shen et al., 2025) (Figure 2B).

CRACD is an important regulator of actin dynamics that promotes F-actin formation by inhibiting the actin capping proteins CAPZA and CAPZB. In small-cell lung cancer, CRACD loss disrupts actin organization and alters YAP1-dependent NOTCH signaling, affecting ASCL1 expression, neuroendocrine plasticity, and tumor heterogeneity (Seo et al., 2025). Actin functions extend beyond the cytoplasm, as nuclear actin contributes to chromatin organization, transcription, RNA processing, and DNA repair (Huang et al., 2022) (Figure 2C). Loss of nuclear actin associated with CRACD deficiency may enhance the activity of the PRC2 catalytic subunit EZH2, increasing H3K27me3 levels and suppressing gene transcription. In particular, repression of MHC-I genes can reduce antigen presentation to $CD8^+$ T cells and promote immune evasion, whereas EZH2 inhibition may restore MHC-I expression and $CD8^+$ T-cell infiltration (Seo et al., 2025) (Figure 2D). These findings highlight a link between actin organization and epigenetic regulation.

Disruptions in actin organization are associated not only with cancer but also with neurodegenerative diseases, cardiomyopathies, muscular dystrophies, renal disorders, and developmental abnormalities (Parker et al., 2020). Given the roles of actin filaments in spermatogenesis, oocyte maturation, meiotic spindle formation, fertilization, and early embryonic development, altered actin dynamics may also contribute to infertility and embryonic developmental defects (Santella et al., 2020; Soda et al., 2020). Moreover, oxidative stress and chronic inflammation associated with diabetes, obesity, and aging can further disrupt actin organization, contributing to pathological changes at the cellular and tissue levels (Stojanovic et al., 2025; Zhao et al., 2026) **(Figure 2).**

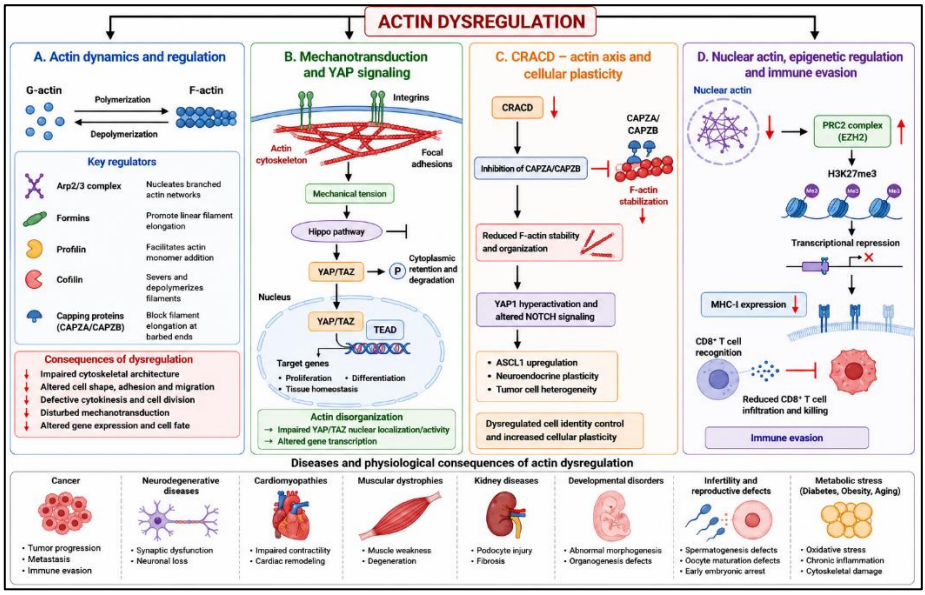



**Figure 2. Effects of Disrupted Actin Dynamics on Cellular Signaling, Epigenetic Regulation, and Cellular Plasticity. (A)** The G-actin/F-actin transition is regulated by key actin-associated proteins, and its disruption can affect cytoskeletal organization, adhesion, cytokinesis, and cell fate. **(B)** Actin-generated tension regulates the Hippo-YAP/TAZ pathway, linking cytoskeletal dynamics to proliferation and differentiation. **(C)** CRACD loss disrupts F-actin organization and alters YAP1/NOTCH signaling, ASCL1 expression, and neuroendocrine plasticity. **(D)** Reduced nuclear actin may enhance PRC2/EZH2 activity and H3K27me3 levels, suppressing transcription and MHC-I expression and thereby promoting immune evasion. Actin dysfunction is also associated with neurodegenerative, cardiac, muscular, renal, developmental, and reproductive disorders. Figure created using ChatGPT (OpenAI), based on author-defined content, and subsequently refined and verified by the authors.

### 3.2. Microtubule dysfunction

Microtubules are composed of α- and β-tubulin heterodimers and are essential for maintaining cell shape, organelle transport, mitotic spindle formation, and chromosome segregation (Pais & Schou, 2026). Their dynamic balance between polymerization and depolymerization is regulated by tubulin isotypes, post-translational modifications, and microtubule-associated proteins (Pais & Schou, 2026). Microtubule dysfunction can result from mutations in tubulin genes, defects in microtubule-associated proteins, or impaired regulation of microtubule dynamics. Pathogenic variants in tubulin genes, including TUBA1A, TUBB2A, TUBB2B, TUBB3, and TUBB4A, have been associated with a range of

neurodevelopmental phenotypes, including neuronal migration defects and cortical malformations. The clinical consequences vary according to the affected gene and variant. Other tubulin genes should be discussed separately when their association with developmental phenotypes is supported by specific evidence (Sferra et al., 2020).

In neurons, microtubules are essential for axonal transport, synaptic vesicle trafficking, and mitochondrial distribution. Accordingly, microtubule dysfunction is associated with neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, ALS, and frontotemporal dementia (Sferra et al., 2020; Zhang et al., 2022). In Alzheimer's disease, amyloid-β- and tau-related processes can disrupt microtubule stability and axonal transport (Chinnathambi & Adityan, 2026). Defects in mitotic spindle organization may cause aneuploidy and genomic instability, whereas disruption of ciliary microtubules is associated with primary ciliary dyskinesia, infertility, and developmental abnormalities (Gunes et al., 2020; Parrella et al., 2026). Impaired intracellular transport may also contribute to mitochondrial dysfunction, oxidative stress, and endoplasmic reticulum stress. Thus, tubulinopathies, lissencephaly, polymicrogyria, microcephaly, neurodegenerative disorders, certain cancers, and primary ciliary dyskinesia are among the major pathologies associated with microtubule dysfunction (Klemmensen et al., 2024; Shen et al., 2025). These dynamic properties and the major cellular and pathological consequences of microtubule dysfunction are summarized in Figure 3.

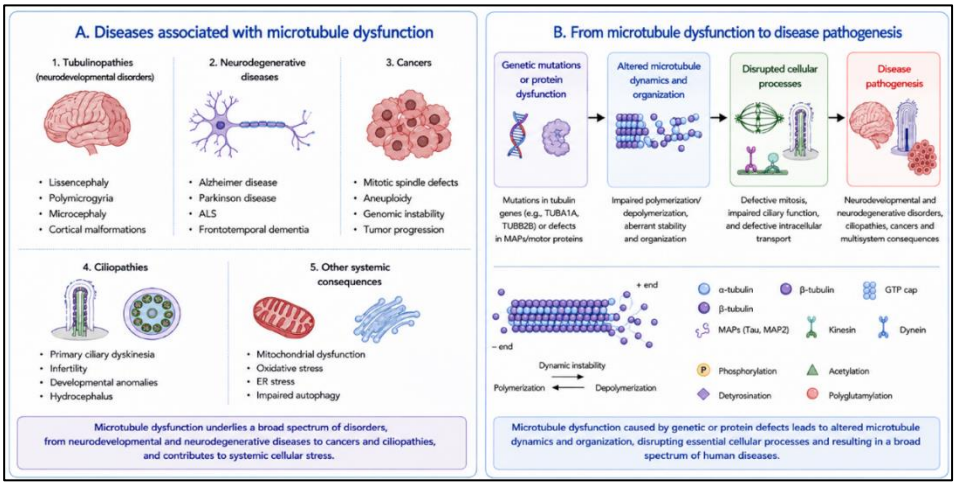



**Figure 3.** Effects of Microtubule Dysfunction on Cellular Processes and Disease Development. Figure created using ChatGPT (OpenAI), based on author-defined content, and subsequently refined and verified by the authors.

### 3.3 Intermediate filament abnormalities

Intermediate filament disorders arise from gene mutations, deletions, or alterations in filament organization. These defects can compromise filament structure and cellular mechanical resilience, leading to impaired tissue integrity and abnormal protein accumulation (Gao & Nakamura, 2025).

**Table 1.** Core Pathological Mechanisms and Associated Diseases of Intermediate Filament Disorders by Filament Type

| Affected intermediate filament group | Primary pathological mechanism | Characteristic clinical entity |
|---|---|---|
| Keratins (Type I/II) | Disruption of the filament network and cell lysis | Epidermolysis Bullosa Simplex (EBS) |
| Desmin (Type III intermediate filament) | Disruption of myofibril organization and protein accumulation | Desminopathy |
| Plectin (Intermediate filament-associated linker protein) | Impairment of cytoskeletal links and mechanical integrity | Plectin-related myopathies and certain forms of epidermolysis bullosa simplex |
| Neurofilaments (Type IV) | Impaired axonal transport and protein accumulation | Neurological disorders associated with neurofilament defects |
| Lamins (Type V) | Disruption of nuclear envelope and chromatin organization | Emery-Dreifuss muscular dystrophy, progeria. |

One of the best characterized keratinopathies is Epidermolysis Bullosa Simplex. Mutations in KRT5 and KRT14 disrupt the mechanical stability of the keratin network in basal keratinocytes, leading to cell lysis and blister formation. The keratin network also contributes to cellular organization and protection against UV-induced DNA damage (Chen et al., 2021). In muscle tissue, desmin and plectin support myofibrillar organization and mechanical force transmission. DES mutations and defective desmin filament organization can cause protein accumulation and myofibrillar abnormalities, resulting in desminopathies characterized by muscle

weakness, cardiomyopathy, and cardiac conduction defects (Maggi et al., 2021). Neurofilament abnormalities can impair axonal transport and promote protein accumulation, contributing to the progression of neurodegenerative disorders such as ALS (Lefebvre-Omar et al., 2023) (Table 1).

## 4. Cytoskeletal Dysfunction in Developmental Disorders

### 4.1. Neurodevelopmental Disorders: Lissencephaly, Microcephaly, and Neuronal Migration Defects

The development of the mammalian cerebral cortex requires the precise coordination of neural progenitor cell proliferation, neuronal migration, cellular polarization, axon guidance, and synaptic maturation. These processes rely heavily on microtubules, actin filaments, and associated motor proteins (Peregrina & Del Toro, 2020). During corticogenesis, cytoskeleton-dependent mechanisms govern neuronal migration, leading-process formation, centrosome positioning, and nucleokinesis. Accordingly, defects in cytoskeletal organization can disrupt cortical architecture and neuronal connectivity, contributing to a range of neurodevelopmental disorders (Achkasova et al., 2026).

Lissencephaly is one of the best-characterized examples and is characterized by agyria or pachygyria, cortical thickening, and impaired neuronal migration. Mutations in LIS1 (PAFAH1B1) disrupt dynein function, affecting centrosome positioning and nucleokinesis, whereas alterations in DCX and TUBA1A impair microtubule organization and neuronal migration, resulting in lissencephaly and related cortical malformations (Di Donato et al., 2017; Ortug et al., 2023).

Primary microcephaly arises from insufficient expansion of the neural progenitor cell pool. Mutations in genes involved in centrosome function and mitotic spindle organization, including ASPM, WDR62, CDK5RAP2, CEP152, and STIL, disrupt progenitor cell division and restrict cortical growth (Ribeiro et al., 2025). Alterations in genes such as WDR62 and TUBA1A may additionally affect neuronal migration, resulting in microcephaly accompanied by cortical malformations (Dell'Amico et al., 2023).

Cytoskeletal components and associated signaling pathways also contribute to other cortical developmental disorders, including subcortical band heterotopia, polymicrogyria, and agenesis of the corpus callosum. ARX mutations impair the migration of GABAergic interneurons, whereas defects in RELN and VLDLR disrupt Reelin signaling and interfere with the proper positioning of neurons within cortical layers (Tsuboi & Yoshihara, 2025). Furthermore, abnormalities in dynein- and kinesin-mediated microtubule transport can impair organelle and vesicle trafficking, thereby affecting axonal development and synaptic maturation (Qu et al., 2025). Collectively, these findings indicate that defects in microtubule and actin

dynamics, centrosomal organization, and motor protein function represent common molecular mechanisms underlying neurodevelopmental disorders (Figure 4A).

### 4.2. Congenital brain malformations

Congenital brain malformations are a heterogeneous group of disorders that arise during embryonic development and disrupt the structural organization of the brain (AlRayahi et al., 2026). Defects in neural progenitor cell proliferation, differentiation, neuronal migration, and cortical organization represent major underlying mechanisms. Because the cytoskeleton is essential to these processes, mutations in cytoskeleton-related genes can cause a broad spectrum of cortical malformations (Achkasova et al., 2026).

During cerebral cortex development, radial glial cells maintain the neural progenitor pool and guide newly generated neurons toward appropriate cortical layers. Actin filaments regulate cell shape, adhesion, and migration, whereas microtubules contribute to cell polarity, mitosis, nuclear movement, and axonal extension. Disrupting these mechanisms can cause microcephaly, macrocephaly, lissencephaly, and periventricular nodular heterotopia (Ferent et al., 2020).

In microcephaly, mutations in CDK5RAP2, ASPM, WDR62, and CPAP impair neural progenitor proliferation and restrict brain growth, whereas alterations in PTEN, RAB39B, CHD8, and STRADA are associated with macrocephaly (Rasool et al., 2020; Yeter et al., 2025). In lissencephaly, LIS1, DCX, and TUBA1A mutations disrupt microtubule organization and dynein-dependent neuronal migration (Aiken et al., 2019). In periventricular nodular heterotopia, alterations in DCHS1, FAT4, ECE2, and GNG5 may affect cell adhesion, actin organization, and microtubule dynamics (Song et al., 2026).

Brain organoids derived from human pluripotent stem cells provide valuable models for investigating human-specific mechanisms underlying these disorders. Advances in single-cell omics, genome editing, and organoid technologies are enabling more detailed investigation of the cellular and molecular mechanisms underlying congenital brain malformations (Kim et al., 2026; Li et al., 2025) (Figure 4B).

### 4.3. Craniofacial Disorders: Neural Crest Cell Migration Defects

Craniofacial development is a complex process that requires coordinated cell migration, proliferation, and differentiation (Roth et al., 2021). A substantial portion of the head and facial structures derives from cranial neural crest cells (CNCCs), which migrate from the neural tube to the developing craniofacial region. Accordingly, defects in CNCC formation, epithelial-mesenchymal transition (EMT), migration, or differentiation can cause craniofacial anomalies (Rigueur & Boughner,

2025). CNCC migration and EMT are regulated by BMP, FGF, Wnt, and Notch signaling, together with interactions among cell adhesion molecules (Zhu et al., 2026), the cytoskeleton, and extracellular matrix. Actin remodeling is particularly important for cell shape and motility, while transcription factors such as Snail, Twist, Sox9, Sox10, and FoxD3 promote migratory properties (Ji et al., 2019). Impaired CNCC migration or proliferation can affect the development of the mandible, maxilla, palate, and cranial bones, leading to conditions such as Treacher Collins syndrome, cleft palate, and Pierre Robin sequence. For example, TCOF1 mutations increase cell loss in neural crest progenitors and impair craniofacial development (Siismets & Hatch, 2020) (Figure 4C).

Defects in post-migratory neural crest development can cause skeletal abnormalities such as craniosynostosis (Siismets & Hatch, 2020). Alterations in FGFR1/2/3, TWIST1, and MSX2 affect osteoblast differentiation and cranial suture development, while epigenetic regulators such as KMT2D and EZH2 contribute to neural crest cell fate and osteogenic differentiation (Liao et al., 2022). These findings indicate that craniofacial development is shaped by the coordinated interplay of cytoskeletal dynamics, cell migration, signaling pathways, and epigenetic regulation.

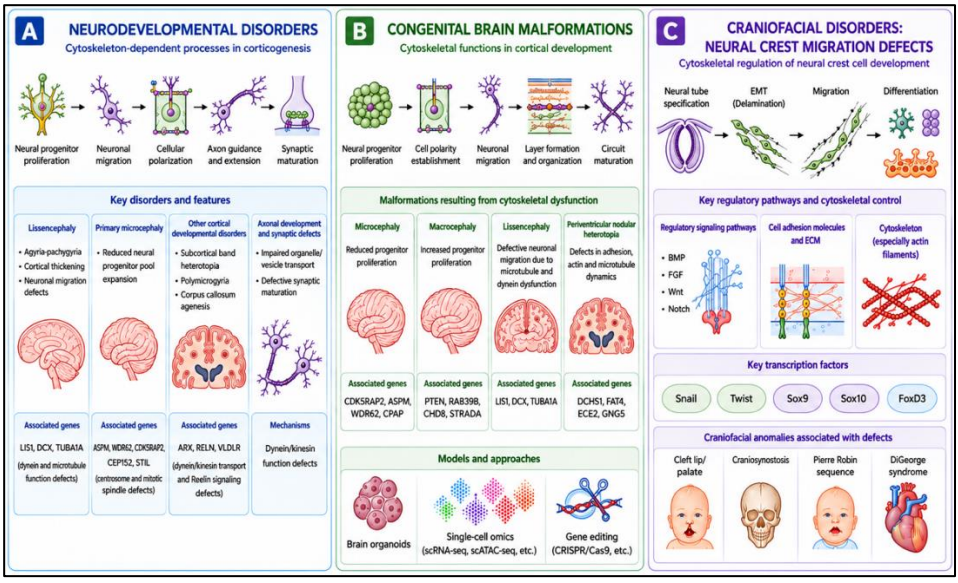



**Figure 4. Cytoskeletal mechanisms underlying neurodevelopmental, congenital brain, and craniofacial disorders.** The figure summarizes the major cytoskeleton-dependent cellular processes, associated developmental abnormalities, key genes and regulatory pathways involved in neurodevelopmental (A), congenital brain (B) and craniofacial development (C),

and the principal experimental approaches used to investigate these mechanisms. Figure created using ChatGPT (OpenAI), based on author-defined content, and subsequently refined and verified by the authors.

### 4.4. Cardiac developmental defects

Cardiac development occurs through the coordinated progression of interconnected processes, including cardiac progenitor differentiation, heart tube formation, ventricular trabeculation, myocardial maturation, and cardiomyocyte organization (Byatt et al., 2025). The cytoskeleton, cell adhesion molecules, extracellular matrix (ECM), and contractile proteins function together during these processes, and their disruption can contribute to congenital heart defects and cardiomyopathies (Wang et al., 2025).

Dystroglycan (DG) is an important component of the cytoskeleton-ECM interface, forming a mechanical link between the ECM and the actin cytoskeleton (Hopkinson & Pitsillides, 2025). Mutations in genes involved in α-dystroglycan glycosylation, including POMT1, POMT2, POMGNT1, POMK, FKTN, FKRP, B4GAT1, and LARGE1, can disrupt this connection and cause dystroglycanopathies affecting cardiac function (Sharaf-Eldin, 2025). Alterations in actin and sarcomeric proteins can also impair cardiomyocyte organization and contractile function; mutations in ACTC1, MYH6, MYH7, TNNT2, TNNI3, TPM1, and MYBPC3 are associated with congenital heart defects and cardiomyopathies (Barefield et al., 2023).

In addition, alterations in the RNA-splicing factor PRPF8 and the transcription factor TEAD1, as well as dysregulation of the YAP/TEAD and Notch1 signaling pathways, may affect myocardial organization, cardiomyocyte differentiation, and ventricular development (Liu et al., 2025; Song et al., 2024). These data indicate that cardiac developmental defects are associated with disruptions in cytoskeleton-ECM interactions, sarcomeric proteins, RNA processing, and developmental signaling networks (Figure 5A).

### 4.5. Muscle Disorders and Muscular Dystrophies

Musculoskeletal disorders comprise a heterogeneous group of diseases that affect the structural integrity, contractility, and mechanical resilience of muscle fibers (Cardia et al., 2026). Normal muscle function depends on the coordinated interaction of actin-myosin-based sarcomeres, the cytoskeleton, membrane-associated complexes, and the ECM. Disruption of these components can lead to congenital myopathies and muscular dystrophies (Campuzano-Donoso et al., 2026).

Actin contributes to contractile force generation, microtubules to myofibril organization and intracellular transport, and desmin to myofibril alignment and

mechanical integrity. Mutations in ACTA1, ACTC1, MYH7, MYBPC1/3, TTN, NEB, and TPM2 can disrupt sarcomere organization and contractile function, leading to various myopathies (Chong et al., 2023; Clayton et al., 2024). In Duchenne muscular dystrophy, dystrophin loss caused by DMD mutations weakens the actin-ECM connection and promotes progressive muscle fiber degeneration (Sarvutiene et al., 2025).

Disruption of dystroglycan- and integrin-mediated interactions also compromises muscle-ECM integrity. Mutations in POMT1, POMT2, POMGNT1, POMK, FKTN, FKRP, B4GAT1, and LARGE1 can cause dystroglycanopathies, including Walker-Warburg syndrome, Muscle-Eye-Brain disease, and Fukuyama congenital muscular dystrophy (Sharaf-Eldin, 2025). Notch, Wnt/β-catenin, and MyoD/Myf5 pathways regulate muscle development and regeneration, whereas NF-κB and TGF-β/SMAD signaling contribute to inflammation and fibrosis during chronic injury (Li et al., 2026) (Figure 5B).

### 4.6. Kidney developmental defects

Kidney development depends on the coordinated interaction between the ureteric bud and metanephric mesenchyme, involving signaling, cell differentiation, polarization, and tubular morphogenesis (Khoshdel Rad et al., 2020). Disruptions in these processes can lead to congenital anomalies of the kidney and urinary tract (CAKUT), including renal agenesis, hypoplasia, and dysplasia (Walawender et al., 2023).

The GDNF-RET, WNT, BMP, and FGF pathways regulate renal organogenesis, while PAX2, WT1, SIX2, and HNF1B contribute to nephrogenic progenitor maintenance and differentiation. Alterations in these genes are associated with renal agenesis, hypoplasia, dysplasia, and various CAKUT phenotypes (Fourikou & Dotis, 2025). Actin and microtubule organization further regulate cell polarity, adhesion, cell shape, and tubular elongation, while dysregulation of the WNT/β-catenin, Notch, and Hippo-YAP/TEAD pathways can affect proliferation, differentiation, and kidney development (Ajongbolo & Langhans, 2025; Zhang et al., 2026) (Figure 5C).

Renal organoids derived from human pluripotent stem cells have emerged as valuable models for investigating kidney development and the molecular mechanisms underlying congenital renal disorders (Kang, 2023). These approaches provide important opportunities to elucidate the roles of developmental genes and cellular organization mechanisms in human kidney development.

### 4.7. Ciliopathies

Cilia are microtubule-based structures involved in cellular sensing, cell polarity, and developmental signaling. Structural or functional defects in cilia can cause

ciliopathies affecting the kidneys, retina, brain, respiratory system, and reproductive organs (Cajanek et al., 2025). Ciliary function depends on the coordinated activity of the basal body, axoneme, intraflagellar transport (IFT) system, and kinesin/dynein motor proteins (Li & Ran, 2024).

Primary cilia regulate several developmental signaling pathways, particularly Hedgehog, as well as WNT, BMP, and TGF-β pathways. Consequently, ciliary defects can disrupt organ development and cell differentiation (Anvarian et al., 2019). Mutations in *PKD1, PKD2,* and *NPHP* genes are associated with renal ciliopathies, including polycystic kidney disease and nephronophthisis (Adamiok-Ostrowska & Piekielko-Witkowska, 2020). In Joubert, Meckel-Gruber, and Bardet-Biedl syndromes, defects in ciliary biogenesis, protein trafficking, or signaling can lead to neurodevelopmental and retinal abnormalities (Van De Weghe et al., 2022). Similarly, dysfunction of motile cilia underlies primary ciliary dyskinesia, which is characterized by recurrent respiratory tract infections, bronchiectasis, and infertility. In addition, impaired nodal cilia function can disrupt left-right body patterning during embryonic development, leading to laterality defects such as situs inversus (Despotes et al., 2024) (Figure 5D).

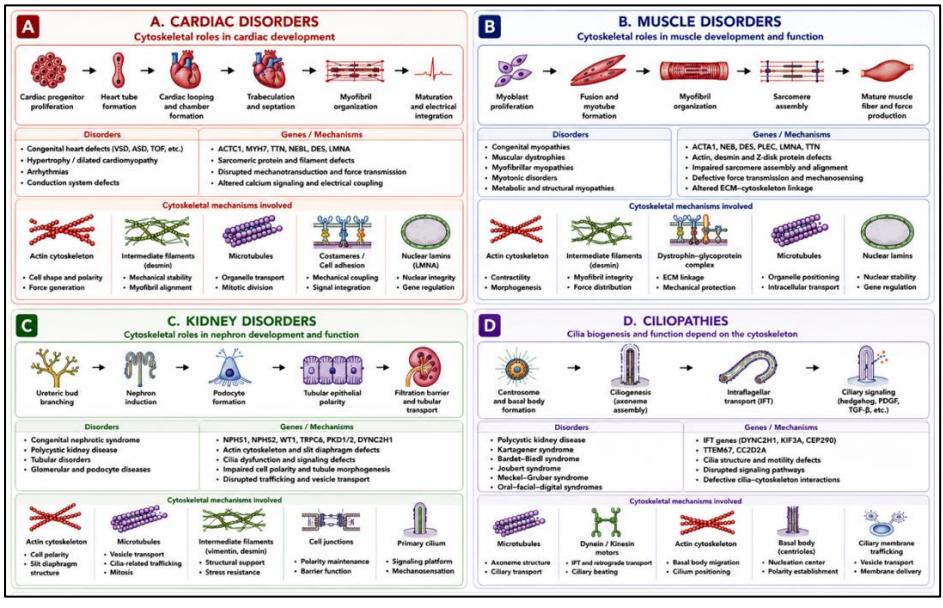



**Figure 5. Cytoskeletal mechanisms underlying cardiac, muscle, kidney, and ciliary disorders.** The figure summarizes the roles of cytoskeletal components in cardiac development and function, muscle development and integrity, nephron development and tubular organization, and ciliogenesis and ciliary signaling, highlighting associated disorders, key genes and molecular mechanisms, and the

major cytoskeletal structures involved in these processes. Figure created using ChatGPT (OpenAI), based on author-defined content, and subsequently refined and verified by the authors.

## 5. Future Perspectives

A more detailed understanding of the cytoskeleton's role during embryonic development will require integrating different research approaches. Single-cell and spatial omics technologies can help reveal how cytoskeletal changes vary among cell types and developmental stages, while advanced imaging approaches allow these changes to be examined over time. Organoid and organ-on-chip models also provide more controlled settings for investigating the effects of cytoskeletal dysfunction on cell behavior, tissue organization, and organ development.

Combining live-cell imaging, quantitative analysis, and CRISPR/Cas-based gene editing can further clarify the relationships between cytoskeletal alterations and developmental phenotypes. In addition, integrating molecular, cellular, and biomechanical data may better clarify the cytoskeleton as more than a structural support system, highlighting its role as a dynamic system that converts mechanical cues into cellular signals. This perspective may clarify the mechanisms underlying developmental disorders associated with cytoskeletal dysfunction and help identify new diagnostic and therapeutic targets.

## 6. Conclusion

The cytoskeleton is a dynamic and integrated system that regulates cellular behavior and tissue organization throughout embryonic development. The coordinated functions of actin filaments, microtubules, and intermediate filaments support developmental processes such as cell polarity, proliferation, migration, morphogenesis, and organogenesis. These processes are closely linked to cell–cell and cell–extracellular matrix interactions, intracellular organization, and the transmission of mechanical signals.

Alterations in cytoskeletal components or their regulatory mechanisms can affect not only cellular functions but also the structural and functional properties of developing tissues. Disorders affecting neuronal, craniofacial, cardiac, musculoskeletal, renal, and ciliary development illustrate the broad importance of cytoskeletal regulation across different developmental systems. Despite their diverse clinical manifestations, these disorders share disturbances in cellular organization, cell movement, force regulation, or tissue architecture. The cytoskeleton's structural, mechanical, and regulatory functions are not independent; they operate as interconnected processes throughout development.

Therefore, maintaining cytoskeletal integrity is essential for the proper progression of embryonic development, from cellular organization to tissue and organ formation.

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# Chapter 7

## Cytoskeletal Alterations in Major Neurodegenerative Proteinopathies

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**Abstract**

Microtubules, actin filaments, neurofilaments and their associated motors and regulatory proteins interact to coordinate neuronal structure and function. Increasingly, disruption of these systems is recognized as a convergent mechanism in major neurodegenerative proteinopathies. In this chapter, we review the normal organization and developmental functions of the neuronal cytoskeleton and discuss the effects of tau, α-synuclein, TDP-43 and huntingtin pathology on cytoskeletal dynamics in Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis/frontotemporal dementia and Huntington's disease. Special focus is on axonal transport failure, synaptic actin remodeling, neurofilament pathology, diagnostic biomarkers and experimental models. Contemporary cytoskeleton-targeting therapies have yielded robust preclinical signals but limited clinical efficacy, highlighting the need for earlier intervention, biomarker-guided trial design and human-cell models that retain disease-relevant genetic backgrounds.

**Keywords:** neuronal cytoskeleton; microtubules, actin; neurofilaments; tau; α-synuclein; TDP-43; Huntingtin, neurodegeneration

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## 1. Introduction

A eukaryotic cell is shaped, mechanically resilient, and capable of directed, polarized function because of its internal scaffold, the cytoskeleton. In neurons, this scaffolding is elaborated to an extraordinary degree: a single cell body may support an axon extending well over a meter in length and a dendritic arbor receiving thousands of synaptic contacts, all built and maintained by three interacting filament systems, microtubules, actin microfilaments, and neurofilaments (intermediate filaments), together with the motor proteins, cross-linkers, and regulatory kinases that act upon them (Muñoz-Lasso et al., 2020; Richardson et al., 2024). Neurons are post-mitotic cells which must maintain axonal transport, synaptic architecture and structural integrity for a human life, making them unusually dependent on continuous cytoskeletal turnover and repair and unusually vulnerable when those turnover fails.

Over the past two decades, cytoskeletal dysfunction has emerged as a common mechanistic feature of several major neurodegenerative proteinopathies. The pathological hallmarks of Alzheimer's disease (AD) are extracellular amyloid-β (Aβ) plaques and intraneuronal neurofibrillary tangles composed of the microtubule-associated protein tau, the latter being the component most directly linked to cytoskeletal disruption. In Parkinson's disease (PD) and the other synucleinopathies, α-synuclein species directly disrupt actin and microtubule dynamics. In amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), TDP-43 proteinopathy is accompanied by neurofilament accumulation and impaired axonal transport. In Huntington's disease (HD), the normal function of huntingtin protein as an actin- and microtubule-associated scaffold is disrupted by polyglutamine expansion (Brill et al., 2024). These are mechanistically distinct proteins that cause clinically distinct diseases, but in each case the neuronal cytoskeleton is both an early casualty and an active participant in the propagation of pathology.

This chapter addresses cytoskeletal changes in neurodegenerative disease from several complementary directions. It starts with the normal histology of the neuronal cytoskeleton and its embryological development and continues to examine the molecular, histological and cellular structure of the major disease-associated proteins and the major signaling pathways regulating them in normal conditions. It then discusses how these systems break down in disease, the resulting histopathological and structural changes, and the clinical manifestations and diagnostic biomarkers these changes produce. The chapter concludes with a summary of the experimental models studied for these processes, the therapeutic approaches developed to date, future perspectives, and a synthesis of the evidence.

## 2. Normal Histology and Embryological Development

The neuronal cytoskeleton comprises three major polymer systems that differ in subunit chemistry, diameter, mechanical properties, and function, but are extensively cross-linked and functionally interdependent, and are themselves products of a tightly choreographed embryological program (Kotaich et al., 2023; Muñoz-Lasso et al., 2020).

### 2.1 Microtubules

Microtubules are hollow polymers of 25 nm diameter consisting of heterodimers of α- and β-tubulin polymerized head-to-tail into 13 protofilaments. They have an intrinsic polarity with a fast growing plus end and a slower minus end that supports dynamic instability and their use as directional tracks for kinesin and dynein motors that ferry organelles, vesicles, mRNA granules and mitochondria in anterograde and retrograde directions (Dutta et al., 2018). Axonal microtubules are predominantly arranged with plus ends outwards, whereas dendritic microtubules are of mixed polarity in mammalian neurons. This arrangement is set up with axon specification and forms the basis for polarized cargo sorting (Yau et al., 2016). Microtubule-associated proteins (MAPs) affect lattice stability, spacing and bundling; tau in axons and MAP2 in dendrites are the most extensively studied. Phosphorylation-dependent regulation of MAP binding is a key factor in the pathogenesis of several neurodegenerative diseases (Richardson et al., 2024).

### 2.2 Actin Microfilaments

Nucleators (the Arp2/3 complex, formins) and severing/depolymerizing factors (cofilin, gelsolin) organize actin into branched networks, linear bundles, and cortical meshworks, in a dynamic equilibrium between monomeric G-actin and filamentous F-actin. In neurons F-actin is enriched at the growth cone during development and, in the mature cell, at the presynaptic terminal and in dendritic spines, where activity-dependent actin remodeling underlies structural plasticity and synaptic strength (Da Silva & Dotti, 2002; Wurz et al., 2022). Cofilin-actin rods, rigid bundles that occlude dendritic actin dynamics and are increasingly recognized in multiple neurodegenerative conditions (Wurz et al., 2022) can be driven by disruption of the cofilin-actin regulatory cycle, for example by oxidative or metabolic stress.

### 2.3 Neurofilaments and the Intermediate Filament Network

Neurofilaments are neuron-specific type IV intermediate filaments, composed of light (NF-L), medium (NF-M) and heavy (NF-H) subunits, along with α-

internexin in the central nervous system and peripherin in the peripheral nervous system (Kotaich et al., 2023; van Asperen et al., 2024). Neurofilaments are relatively stable, slowly transported structural polymers whose main function is to provide axonal caliber. Radial growth of large-diameter myelinated axons is dependent on neurofilament side-arm cross-bridging. Because neurofilament subunits are released into extracellular fluid in proportion to the degree of axonal damage after injury, they have become one of the most useful fluid biomarkers of neuroaxonal injury across neurological diseases, as discussed in the clinical manifestations and biomarkers section below (van Asperen et al., 2024). Together with these three core polymers, the sub-membranous spectrin–actin lattice contributes to axonal mechanical stability and the membrane-associated periodic skeleton, where circumferential actin rings are connected by spectrin tetramers and visualized by super-resolution microscopy (Vassilopoulos et al., 2019). Mutations affecting spectrin isoforms such as β-III spectrin (SPTBN2) produce cerebellar and axonal phenotypes that illustrate how defects of the membrane cytoskeletal can also drive neurodegeneration (Sancho et al., 2021).

### 2.4 Embryological Development of the Neuronal Cytoskeleton

The mature neuronal cytoskeleton described above is the culmination of a developmental program in which microtubule and actin dynamics coordinate nearly every phase of nervous system development. A growing body of evidence suggests that the same cytoskeletal machinery that is disrupted in adult neurodegeneration is initially deployed and vulnerable during embryogenesis (Achkasova et al., 2026).

Neural progenitor cells in the neuroepithelium undergo interkinetic nuclear migration, a process that involves microtubules and dynein and involves movement of the nucleus between the apical and basal surfaces of the ventricular zone in harmony with the cell cycle. After the last mitotic division, newly formed neurons migrate away from the ventricular zone along radial glial fibers. This movement requires coordinated actin-based leading-process protrusion and microtubule-based nucleokinesis, in which the nucleus moves forward through a cage of perinuclear microtubules pulled by cytoplasmic dynein anchored at the centrosome (Lasser et al., 2018). Mutations within the genes encoding this dynein motor complex (DYNC1H1) or its regulatory partners lead to a spectrum of malformations of cortical development, exemplifying the direct consequences of cytoskeletal motor function on brain architecture and subsequently, axonal transport in the adult brain (Becker et al., 2020).

When the neuron reaches its final destination, actin-rich growth cones at the tips of extending processes are used to scan the local environment, translating

extracellular guidance cues into asymmetric actin polymerization that specifies the process that will become the axon, a decision that is underpinned by local microtubule stabilization and enrichment of microtubule-associated proteins within the nascent axon shaft (Da Silva & Dotti, 2002). Subsequent dendritic arborization depends on analogous but spatially distinct cycles of actin- and microtubule-based branching, whereas synaptogenesis requires activity-dependent actin remodeling in nascent dendritic spines. Mutations in cytoskeletal motor and structural genes (DYNC1H1, KIF1A, SPTBN2) produce phenotypes that span a continuum from neurodevelopmental malformation to later-onset hereditary neurodegeneration, depending on the specific domain affected and the severity of the resulting dysfunction (Becker et al., 2020; Dutta et al., 2018; Sancho et al., 2021). This is because the same kinesin and dynein motors that drive interkinetic nuclear migration and nucleokinesis later become the principal long-range transport engines of the mature axon. This developmental framing suggests that at least some susceptibility to adult-onset cytoskeletal collapse may be seeded by subtle, subclinical developmental variation in cytoskeletal gene dosage or regulation, but this is an area of active investigation rather than an established mechanism.

## 3. Molecular Organization and Major Cytoskeletal Signaling Pathways

Four proteins, tau, α-synuclein, TDP-43 and huntingtin, provide a useful framework to compare cytoskeletal dysfunction in the major proteinopathies discussed here. Tau and huntingtin directly bind to the cytoskeletal polymers. α-synuclein regulates actin and microtubule dynamics, and TDP-43 affects cytoskeletal homeostasis mainly by RNA processing, nucleocytoplasmic transport and subsequent changes in axonal proteins. In this context, understanding these normal and pathological relationships provides a basis for comparing disease mechanisms, without presupposing that all four proteins are canonical cytoskeletal components.

### 3.1 Tau and the GSK-3β/CDK5 Signaling Axis (Alzheimer's Disease)

Tau is an intrinsically disordered microtubule-associated protein encoded by MAPT. Under physiological conditions, the C-terminal region of tau contains three or four imperfect microtubule-binding repeat domains that bind along the outer surface of the microtubule lattice, promoting assembly and buffering dynamic instability, particularly in unmyelinated and thin axons. The tau function is tightly regulated by phosphorylation at more than 80 serine/threonine residues, physiologically balanced by kinases such as glycogen synthase kinase-3β (GSK-3β) and cyclin-dependent kinase 5 (CDK5) and counteracting phosphatases such as

PP2A. This kinase–phosphatase balance tunes tau's affinity for microtubules according to the neuron's metabolic and developmental state (Basheer et al., 2023).

### 3.2 α-Synuclein, Cytoskeletal Interactions and Pathological AMPK–p38 MAPK Signaling

α-Synuclein (α-Syn) is a small, natively unstructured presynaptic protein whose physiological functions include modulation of synaptic-vesicle clustering and membrane curvature to SNARE-complex assembly. It also binds actin and tubulin: its N-terminal amphipathic region binds F-actin, while other regions bind tubulin and modulate microtubule assembly under experimental conditions (Carnwath et al., 2018; Oliveira da Silva & Liz, 2020). Such structural interactions should be differentiated from the disease-associated AMPK/p38 MAPK response. Pathological α-synuclein models exhibit reduced PIKE signaling, overactivation of AMPK/p38 MAPK, and decreased kinesin abundance, impairing motor-cargo interaction and axonal transport (Yang et al., 2025). The evidence

cited therefore suggests a pathological signaling mechanism rather than a normal physiological transport axis associated with α-synuclein.

### 3.3 TDP-43 Structure and RNA-Regulatory Signaling (ALS/FTD)

TDP-43 is a predominantly nuclear DNA/RNA binding protein with two RNA-recognition motifs and a C-terminal glycine-rich low-complexity domain that facilitates protein-protein interactions and reversible incorporation into stress granules. TDP-43 normally shuttles between the nucleus and the cytoplasm to regulate pre-mRNA splicing, mRNA stability and stress-granule dynamics (Chou et al., 2018; Verde et al., 2025). This signaling function relies on the maintenance of nuclear localization through active nucleocytoplasmic transport across the nuclear pore complex, a structure that itself is anchored to and functionally coupled with the underlying cytoskeleton (Chou et al., 2018; Verde et al., 2025).

### 3.4 Huntingtin Structure and Cofilin/LIM-Kinase Signaling (Huntington's Disease)

Wild-type huntingtin (HTT) is not only a scaffold for vesicle motor complexes on microtubules but a direct structural component of the actin cytoskeleton. High resolution structural work has shown that the HTT N-terminal HEAT and bridge domains wrap around F-actin and that HTT dimerizes to cross-link parallel actin filaments into ordered bundles, a function important for growth cone architecture and dendritic spine morphology (Carpentier et al., 2025). HTT also interacts with LIM-kinase (LIMK) to regulate cofilin activity and thereby control actin turnover and activity-dependent AMPA receptor recruitment to the postsynaptic density,

positioning HTT as a signaling hub between synaptic activity and actin cytoskeletal remodeling (Wennagel et al., 2022).

## 4. Disease Mechanisms and Histopathological Changes

When the normal structural and signaling relationships described above are disturbed, each disease protein gives rise to a characteristic cascade of cytoskeletal collapse and a corresponding set of histopathological lesions, many of which were first described decades before their molecular basis was understood. The principal lesions and the cytoskeletal components involved in each disease are summarized in Table 1 and are discussed in detail in Sections 4.1–4.4.

### 4.1 Alzheimer's Disease: Tau Hyperphosphorylation and Neurofibrillary Degeneration

In AD, the GSK-3β/CDK5–PP2A balance described in Section 3 is disrupted. GSK-3β and CDK5 become chronically hyperactive, resulting in hyperphosphorylation of tau at pathological epitopes. This hyperphosphorylation significantly reduces tau's microtubule-binding affinity. The somatodendritic compartment accumulates detached, hyperphosphorylated tau, which undergoes a phase transition into a liquid-like condensate. GSK-3β-catalyzed phosphorylation then templates this tau into the cross-β, filamentous conformation characteristic of AD-type paired helical filaments (Chakraborty et al., 2024). This has two consequences for the cytoskeleton. First, the microtubule structure itself is destabilized and understocked with functional MAPs, impairing kinesin/dynein-based transport. Second, the freed tau nucleates further aggregation, promoting further aggregation. Experimental models support the subsequent spread of tau pathology through anatomically connected neuronal networks (Liu et al., 2012), but DeTure and Dickson (2019) provide the neuropathological basis for interpreting the resulting lesions. At the histological level, this process generates neurofibrillary tangles, which are intraneuronal accumulations of paired helical filaments. These filament pairs are twisted and measure 10–13 nm, with a periodic crossover occurring approximately every 80 nm. The regional spread of these tangles thru the hippocampus and neocortex, which is systematically staged by Braak and Braak, is closely correlated with clinical progression (De Ture & Dickson, 2019). Neuritic plaques are composed of dystrophic tau-positive neurites surrounding an amyloid-β core. Other age-related cytoskeletal pathology observed in several tauopathies and synucleinopathies includes granulovacuolar degeneration and Hirano bodies, actin-rich, rod-shaped eosinophilic inclusions preferentially found in hippocampal CA1 pyramidal neurons (Yoshida et al., 2024).

### 4.2 Parkinson's Disease: α-Synuclein-Driven Cytoskeletal Collapse

In Parkinson's disease, the pathological accumulation of aggregated α-synuclein transforms its normal, modest cytoskeletal interactions into overtly disruptive ones. Multimeric α-synuclein species impair tubulin polymerization and destabilize the microtubule network, while oligomeric and fibrillar forms increase actin instability and induce aberrant actin bundling in neurons (Carnwath et al., 2018; Oliveira da Silva & Liz, 2020). These perturbations are mechanistically associated with axonal transport failure. Pathological α-synuclein species chronically activate the AMPK–p38 MAPK stress-signaling axis described above, but in this dysregulated state, the axis impairs motor-protein loading and processivity rather than simply tuning it, resulting in a measurable reduction in both anterograde and retrograde vesicle transport velocity (Yang et al., 2025). Dopaminergic neurons of the substantia nigra have long, thin, highly arborized axons and are more dependent on efficient long-range transport, which may account for their selective vulnerability. Histologically, the aggregated, phosphorylated α-synuclein together with sequestered cytoskeletal and organellar components form Lewy bodies, intracytoplasmic inclusions with a dense hyaline core and a paler halo, and Lewy neurites, the same aggregated material accumulating along axons and dendrites. Recent work stresses that these cytoskeletal and synaptic disturbances precede obvious neurodegeneration by years, suggesting an initiating rather than terminal role in the pathogenic cascade (Calabresi et al., 2023).

### 4.3 ALS/FTD: TDP-43 Mislocalization and Neurofilament Disruption

In most ALS cases and about half of FTD cases, TDP-43 mislocalizes from the nucleus and accumulates in the cytoplasm as hyperphosphorylated, ubiquitinated aggregates. TDP-43 pathology alters nuclear pore morphology and disrupts bidirectional nucleocytoplasmic transport (Chou et al., 2018). Experimental evidence also shows that nuclear loss or cytoplasmic accumulation of TDP-43 can alter the expression and subcellular distribution of neurofilament proteins (Atkinson et al., 2021). These changes are associated with impaired axonal transport, neuromuscular-junction dysfunction and accumulation of phosphorylated neurofilaments in proximal axons (Lépine et al., 2022; Verde et al., 2025). Histologically, TDP-43 proteinopathy results in skein-like or compact cytoplasmic inclusions in spinal and cortical motor neurons. Axonal spheroids with disorganized neurofilaments and stalled organelles are a major marker of axonal transport failure, but they should not be considered a lesion specific to TDP-43 proteinopathy. These findings are consistent with a reciprocal relationship between protein mislocalization, cytoskeletal disruption and transport failure.

### 4.4 Huntington's Disease: Mutant Huntingtin and Actin Cytoskeletal Dysregulation

Polyglutamine expansion in HD converts the normal transport and actin regulatory functions of huntingtin into a source of cytoskeletal dysfunction. Polyglutamine toxicity uncouples dendritic-spine morphology from synaptic activity and impairs cofilin-dependent actin remodeling, resulting in loss of normal HTT function (Wennagel et al., 2022). Mutant HTT also associates with abnormal formation and clearance of nuclear cofilin–actin rods, but these rods are stress-responsive structures found in multiple neurodegenerative diseases, not a lesion specific to HD (Wurz et al., 2022). On the microtubule side, mutant or depleted HTT impairs fast axonal transport of BDNF- and APP-containing vesicles and autophagosomes, exacerbating actin-based deficits and contributing to the vulnerability of striatal medium spiny neurons (Taran et al., 2020). Neuronal intranuclear and cytoplasmic inclusions made of polyglutamine-expanded huntingtin fragments, mainly in striatal and cortical neurons, are histological hallmarks of HD. Independent structural and cellular studies show that wild-type HTT binds and bundles F-actin and regulates cofilin-dependent actin remodeling; these studies provide a mechanistic link to cytoskeletal dysfunction but do not by themselves establish frequent actin/cofilin co-localization within human HD inclusions (Carpentier et al., 2025; Wennagel et al., 2022).

**Table 1.** Principal histopathological lesions linked to cytoskeletal alterations.

| Disease | Principal Lesion | Main Cytoskeletal Component Involved |
|---|---|---|
| Alzheimer's disease | Neurofibrillary tangles; neuritic plaques; Hirano bodies | Microtubules (tau) (DeTure & Dickson, 2019; Yoshida et al., 2024) |
| Parkinson's disease / Dementia with Lewy Bodies (DLB) | Lewy bodies; Lewy neurites | Actin and microtubules (α-synuclein) (Calabresi et al., 2023; Oliveira da Silva & Liz 2020) |
| ALS / FTD | TDP-43 skein inclusions; axonal spheroids | Neurofilaments (associated with TDP-43 dysfunction) (Atkinson et al., 2021; Lépine et al., 2022; Verde et al., 2025) |
| Huntington's disease | Intranuclear and cytoplasmic huntingtin inclusions | Actin and microtubule regulation (huntingtin) (Carpentier et al., 2025; Taran et al., 2020; Wurz et al., 2022) |

## 5. Clinical Manifestations and Diagnostic Biomarkers

Cytoskeletal injury and vulnerability in living patients can be measured directly through the molecular and histopathological cascades mentioned above,

which also translate into distinct but overlapping clinical syndromes and increasingly fluid and genetic biomarkers.

### 5.1 Alzheimer's Disease

Clinically, Alzheimer's disease presents as a progressive amnestic syndrome, with early impairment of episodic memory followed by decline in language, visuospatial function, and executive control as pathology spreads beyond the medial temporal lobe. Because the same underlying tau and amyloid biology can produce different phenotypic presentations (including logopenic, behavioral, or visuospatial variants), clinical presentation alone is not considered diagnostic. The updated criteria of the Alzheimer's Association Workgroup (Jack et al., 2024) explicitly reshape the diagnosis and staging of AD around this biology, integrating blood-based biomarkers with cerebrospinal fluid and imaging measures and delineating a two-dimensional clinical-biological trajectory in which amyloid deposition precedes medial temporal tau accumulation and subsequent cognitive decline.

### 5.2 Parkinson's Disease

PD is clinically defined by bradykinesia in conjunction with rest tremor, rigidity, or both. The core motor requirement under current diagnostic criteria is this bradykinesia-plus-tremor/rigidity combination. We treat postural instability separately as a late, nonspecific feature rather than part of the core diagnostic triad, because its early appearance raises suspicion for an atypical parkinsonian syndrome. Non-motor symptoms including hyposmia, REM-sleep behavior disorder, constipation and depression are often present years before motor onset and are now recognized as a significant, sensitive prodromal signature of the disease, not a late complication (Váradi, 2020). This clinical development aligns with the mechanistic picture outlined in Section 4, which argues that obvious dopaminergic neurodegeneration follows α-synuclein-driven synaptic and cytoskeletal dysfunction (Calabresi et al., 2023).

### 5.3 ALS and Frontotemporal Dementia

ALS is characterized by progressive, asymmetric limb or bulbar weakness, reflecting combined upper and lower motor neuron degeneration. Its clinical spectrum overlaps considerably with FTD, which may present as behavioral disinhibition, apathy or progressive language impairment. Both disorders can be part of a spectrum of TDP-43 proteinopathy. The Gold Coast criteria provided a simple dichotomy for classifying ALS based on progressive motor deficits and the presence of upper and lower motor neuron signs. Several validation studies

have shown higher sensitivity than the revised El Escorial and Awaji criteria, especially for atypical phenotypes, but some clinical and research settings still use the older criteria (Shefner et al., 2020; Timmins et al., 2024).

### 5.4 Huntington's Disease

The classical presentation of HD is a triad of motor, cognitive and psychiatric symptoms. Motor features include chorea (involuntary, irregular movements), loss of manual dexterity, dysarthria and gait instability. Cognitive features start with loss of processing speed and mental flexibility, and progress to global dementia. Critically, cognitive decline and psychiatric symptoms, which are most frequently depression, apathy, and irritability, often precede any observable motor abnormality by up to fifteen years. This clinical timeline is consistent with the early, motor-silent synaptic and actin-cytoskeletal disruption described in Section 4 (Massey & McLauchlan, 2024).

### 5.5 Neurofilament Light Chain as a Cross-Disease Biomarker

Neurofilament light chain (NfL) is released into interstitial fluid, cerebrospinal fluid and blood in proportion to the degree of axonal cytoskeletal injury and is detectable in mutation carriers 10–20 years before estimated clinical onset of autosomal dominant Alzheimer's disease, tracking neurodegeneration well before symptoms emerge, and its trajectory in both CSF and plasma parallels disease progression thereafter (Hofmann et al., 2024). Because NfL elevation is not disease-specific but rather reflects axonal damage broadly, it has been adopted as a pharmacodynamic and prognostic biomarker in AD, ALS, HD, and multiple sclerosis clinical trials, though its lack of specificity and the current lack of fully standardized assay cutoffs are acknowledged limitations of its clinical use (Zheng et al., 2026).

### 5.6 Cytoskeletal Gene Disorders and Genotype–Phenotype Correlations

Cytoskeletal gene mutations also produce informative, if not absolute, correlations between genotype and phenotype. Variants in the N-terminal tail/dimerization region are more frequently associated with neuromuscular and peripheral axonal phenotypes in DYNC1H1-related disorders, whereas variants affecting the motor region are more often associated with neurodevelopmental manifestations, such as cortical development malformations and intellectual disability; however, there is significant phenotypic overlap (Becker et al., 2020). Mutations in the kinesin motor domain of KIF1A can also lead to phenotypes that range from pure hereditary sensory neuropathy to complex spastic paraplegia, with the severity being associated with the degree of impairment of microtubule-stimulated ATPase activity and processive motility (Dutta et al., 2018). Mutations

in SPTBN2, encoding β-III spectrin, cause autosomal-dominant spinocerebellar ataxia type 5 and a spectrum of earlier-onset recessive or de novo phenotypes, revealing a continuum between developmental cytoskeletal defects and later neurodegeneration (Sancho et al., 2021).

## 6. Experimental Models

No single experimental system recapitulates the decades-long evolution, selective neuronal vulnerability, and multicellular context of human neurodegenerative disease. We then investigate cytoskeletal mechanisms using complementary protein-reconstitution, neuronal culture, invertebrate, rodent, and human stem-cell models.

### 6.1 Tauopathy Models

Progressive tau phosphorylation, axonal abnormalities, microtubule loss, and behavioral impairment are replicated in transgenic mouse lines that express mutant human tau, such as PS19 mice. They have played a key role in the screening for brain-penetrant microtubule stabilizers such as epothilone D (Zhang et al., 2012). Models showing region-specific tau expression indicate that tau spreads from the entorhinal cortex to synaptically connected areas in the hippocampus and cortex (Liu et al., 2012). Complementary Drosophila systems enable rapid genetic-modifier screens and separate cell-autonomous toxicity from intercellular tau spread (Bankapalli et al., 2024).

### 6.2 α-Synuclein and Parkinson's Disease Models

α-Synuclein preformed-fibril (PFF) models generate endogenous α-synuclein aggregation by exposing primary neurons or defined brain regions to recombinant fibrils. PFFs induce Lewy body-like pathology, synaptic dysfunction and neuronal death in cultured neurons (Volpicelli-Daley et al., 2011). The SNCA transgenic models cover gene-dosage and mutation-specific effects. In vivo PFF and transneuronal-propagation models allow investigation of the temporal relationship between alpha-synuclein accumulation, axonal transport failure, and dopaminergic degeneration. These systems are experimentally powerful but depend heavily on fibril preparation, injection site, species, and expression level.

### 6.3 TDP-43 and ALS/FTD Models

Models of TDP-43 include inducible cell systems, Drosophila and rodent lines expressing either wild-type or mutant TDP-43, and paradigms that selectively alter nuclear localization. These models discriminate toxic cytoplasmic gain of function from nuclear loss of RNA-regulatory function. For example, Atkinson

et al. (2021) demonstrated that nuclear and cytoplasmic TDP-43 perturbations lead to differential alterations in neurofilament expression and localization. Motor neuron-muscle co-cultures and microfluidic devices allow spatial resolution to study distal axons and neuromuscular junctions, and C9orf72 models include other repeat-RNA and dipeptide-repeat mechanisms not reproduced by TDP-43 overexpression alone.

### 6.4 Huntingtin and Actin Models

In HD, the huntingtin-F-actin complex has been resolved by purified-protein and cellular reconstitution systems, while knock-in and transgenic animals recapitulate selected aspects of polyglutamine-dependent trafficking and behavioral dysfunction (Carpentier et al., 2025; Taran et al., 2020). Live-cell and fixed-tissue assays of cofilin-actin rods provide a tractable readout of stress-induced actin dysregulation, but rod formation is not specific to HD and must be interpreted in combination with disease-relevant HTT phenotypes (Wurz et al., 2022).

### 6.5 Human iPSC-Derived Neurons and Organoids

Patient-derived induced pluripotent stem cell (iPSC) neurons retain disease-associated genetic backgrounds and allow for direct analysis of axonal transport, growth-cone behavior, dendritic spine morphology and cytoskeletal stress in human cells. Isogenic gene-corrected controls minimize background-related confounding and microfluidic culture systems isolate axons from somata. Brain and spinal organoids provide 3-dimensional cell–cell interactions, but are limited by developmental immaturity, variable cellular composition and incomplete recapitulation of aging. Therefore, human-cell models are most useful when used along with reductionist structural systems and in vivo models rather than as replacements.

## 7. Current Therapeutic Approaches

Therapeutic approaches to cytoskeletal protection in neurons can be grouped into direct stabilization of cytoskeletal polymers, inhibition/clearance of disease proteins that damage those polymers, restoration of motor-cargo and actin-regulatory pathways, and biomarker-guided attenuation of ongoing axonal injury. Most direct cytoskeletal approaches remain preclinical; the most advanced clinical experience is with tau-directed and microtubule-directed strategies.

The microtubule-stabilizing agent epothilone D, a brain-penetrant small molecule structurally like the taxanes but with significantly improved blood–brain barrier permeability, was demonstrated to increase microtubule density,

reduce axonal dystrophy, and improve cognitive performance in aged PS19 tau transgenic mice. This resulted in a proof of concept that direct microtubule stabilization can be therapeutically beneficial even after disease onset (Zhang et al., 2012). In progressive supranuclear palsy, a primary tauopathy, a related strategy utilizing a microtubule-stabilizing peptide, davunetide, was advanced to a phase 2/3 randomized, placebo-controlled trial. The trial failed to demonstrate clinical benefit on its primary or secondary endpoints, a result that has been widely discussed as indicative of either an insufficiently potent mechanism, a treatment window that was initiated too late in the disease course, or both (Boxer et al., 2014).

The second approach targets tau aggregation itself. Methylthioninium chloride (and its stabilized reduced form, marketed for trial purposes as LMTM/TRx0237) is a tau aggregation inhibitor that showed encouraging effects in earlier open-label work but failed to separate from a low-dose active comparator arm in a large phase 3 trial in mild-to-moderate AD, again illustrating the difficulty of translating cytoskeleton-protective mechanisms identified in cellular and animal models into clinically detectable benefit at the trial stage (Gauthier et al., 2016). Post-hoc analyzes of the ADAMANT phase 2 trial have suggested a potential slowing of clinical and biomarker decline in participants with both amyloid and tau pathology. This signal will require confirmation in adequately powered, prospectively designed trials (Cullen et al., 2024). More recent work has explored active tau immunotherapy, which is a vaccination strategy that aims to generate antibodies against pathological tau conformers.

With these programs, NfL is being increasingly used as an exploratory, prognostic or pharmacodynamic endpoint, especially in ALS and HD trials, allowing investigators to detect changes in ongoing axonal injury before slower clinical outcomes emerge (Hofmann et al., 2024; Zheng et al., 2026). Experimental approaches for PD include reducing the pathological burden of α-synuclein and blocking downstream stress signaling that impairs kinesin and motor-cargo interactions. Yang et al. (2025) describe a mechanistic target in the AMPK/p38 MAPK findings but have not yet shown clinical efficacy or provided rationale for nonspecific inhibition of the pathway. In ALS/FTD, strategies to restore nuclear TDP-43 function, reverse RNA-processing defects or protect axonal transport are being explored; NfL is a downstream marker of injury but lacks disease specificity. In HD, clinical development of HTT–actin or cofilin pathway modulators lags that of mutant HTT reduction. Direct modulation of huntingtin-actin bundling and cofilin-actin rod formation is still at the structural or preclinical stage (Carpentier et al., 2025; Wurz et al., 2022). Thus, no therapy specifically targeting α-synuclein–actin interactions, TDP-43-dependent

neurofilament changes, huntingtin–actin bundling or cofilin–actin rods has yet shown late-stage clinical efficacy.

## 8. Future Perspectives

Several developments are likely to influence the next phase of research into cytoskeletal alterations in neurodegeneration. First, cryo-electron microscopy and related structural methods are enabling structure-based drug design against cytoskeletal targets previously considered too disordered or dynamic to target rationally, such as the huntingtin-F-actin complex and pathological tau filaments that have already been resolved at atomic resolution (Carpentier et al., 2025). Second, the maturation of fluid biomarkers, particularly neurofilament light chain, but increasingly also phosphorylated tau species and other cytoskeleton-derived fragments, is enabling biomarker-guided, adaptive trial designs that enroll patients earlier in the disease course, consistent with the biology-based staging framework now formalized for AD (Jack et al., 2024) and detect treatment effects on axonal integrity long before clinical endpoints would otherwise be. This is likely to be essential for testing cytoskeleton-stabilizing agents, given the mixed results of earlier trials such as davunetide and LMTM, which may in part reflect intervention initiated too late in the disease process (Boxer et al., 2014; Gauthier et al., 2016; Zheng et al., 2026). Third, gene therapy or gene-modulating techniques developed for monogenic cytoskeletal disorders like DYNC1H1- or KIF1A-related disease may, in theory, inform strategies for the more prevalent, sporadic neurodegenerative diseases in which the same downstream transport machinery is disrupted. However, this translational path is currently speculative (Becker et al., 2020; Dutta et al., 2018). Finally, iPSC-derived neurons and organoids are increasingly being employed in combination with the transgenic and invertebrate models described in Section 6 and provide the opportunity to study cytoskeletal dysfunction in a human genetic background, including in patient-specific mutations, and may help clarify why cytoskeletal collapse in vitro and in animal models does not always correlate with clinical efficacy in human trials. Harnessing this potential will require continued dialog between structural biologists, cell biologists and clinical trialists, since the recurrent lesson of the therapeutic trials reviewed in Section 7 is that a mechanistically-sound cytoskeletal target does not automatically translate into clinical benefit.

## 9. Conclusion

Altering the cytoskeleton is not a peripheral consequence of neurodegenerative disease but rather a central, recurring mechanism. Tau detachment from microtubules, α-synuclein-driven actin and microtubule

dysregulation, TDP-43-associated neurofilament and nuclear pore disruption, or huntingtin's subverted actin-scaffolding function are the three filament systems that form the neuron during embryonic development and whose collapse defines AD, PD, ALS/FTD, and HD in adulthood. These molecular events give rise to each disease's characteristic histopathological lesions, translate into distinct but overlapping clinical syndromes, and are detectable by fluid biomarkers derived from the cytoskeleton (e.g., neurofilament light chain), with direct genotype-phenotype correlations in hereditary cytoskeletal disorders. They are being increasingly precisely modeled in transgenic, invertebrate and structural biology systems. The inconsistent clinical outcomes of therapeutic attempts to directly affect the cytoskeleton, such as microtubule stabilization, aggregation inhibition, or immunotherapy, argue more in favor of earlier intervention, improved biomarker-guided trial design, and ongoing structural and mechanistic research of the type discussed throughout this chapter than against the cytoskeleton as a legitimate target. Taken together the evidence points to the neuronal cytoskeleton as a unifying perspective for understanding the otherwise disparate biology of neurodegenerative disease and from which future treatments are likely to emerge.

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# Chapter 8

## Cytoskeletal Remodeling in Cancer Progression and Metastasis

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**Abstract**

Cytoskeletal remodelling is a dynamic, tightly controlled process that contributes substantially to cancer progression and metastatic dissemination. Beyond its structural role, the cytoskeleton serves as an interface that coordinates mechanical and biochemical cues with cellular polarity, adhesion, migration, invasion, and responses to the tumour microenvironment. This chapter examines the organization and dynamic behavior of actin filaments, microtubules, and intermediate filaments, emphasizing the signaling networks involved in their regulation and plasticity during cancer development. The chapter further addresses how alterations in cytoskeletal organization contribute to epithelial–mesenchymal plasticity, directional migration, extracellular matrix invasion, and mechanotransductive responses. In addition, the chapter critically evaluates current experimental models, cytoskeleton associated molecular biomarkers, and therapeutic strategies targeting cytoskeletal regulators and related signalling pathways. Emerging approaches, including advanced imaging, organoid systems, organ on a chip platforms, and molecular profiling technologies, may further resolve the spatial and temporal complexity of cytoskeletal plasticity and help identify context specific therapeutic vulnerabilities. Overall, understanding cytoskeletal remodeling provides important insights into the mechanisms underlying tumor progression and metastasis and highlights the cytoskeleton as a promising target for translational cancer research.

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## 1. Introduction

Cancer progression is characterised by the acquisition of cellular properties that enable malignant cells to overcome the constraints imposed by the primary tumour and disseminate to anatomically distinct sites. Metastatic dissemination involves a series of coordinated processes, including local invasion, directed migration, interaction with the vascular system, extravasation, and adaptation to distant tissue environments (Mierke, 2026). These processes depend not only on genetic and molecular alterations but also on the ability of tumor cells to dynamically reorganize their morphology, adhesion, contractility, and migratory behavior. In this context, cytoskeletal remodeling represents a fundamental mechanism that enables malignant cells to adapt their structural and functional properties throughout tumor progression and metastasis (Aseervatham, 2020).

Rather than functioning as a static intracellular scaffold, the cytoskeleton is a highly dynamic three-dimensional network that continuously responds to intracellular and extracellular signals. Its major components actin filaments, microtubules, and intermediate filaments cooperate to maintain cellular architecture while regulating intracellular trafficking, polarity, adhesion, and cell movement (Zhou et al., 2026). Beyond their structural functions, cytoskeletal components provide spatial platforms for the organization and localization of signaling molecules, thereby linking cellular architecture to biochemical signaling. Consequently, alterations in cytoskeletal organization can influence both the mechanical behavior of cancer cells and the signaling programs that regulate their responses to the tumor microenvironment (Sevgi et al., 2026).

Cytoskeletal plasticity is particularly important during malignant transformation, when tumor cells acquire enhanced migratory and invasive capacities. Among the cytoskeletal systems, the actin network undergoes rapid cycles of assembly and disassembly that facilitate the formation of specialized membrane structures involved in cell movement and invasion (Keller et al., 2021). Lamellipodia and filopodia contribute to environmental sensing, polarity, and directional migration, whereas

invadopodia facilitate extracellular matrix degradation and tissue invasion. Actin-associated proteins, myosin motors, and multiple signalling pathways regulate the formation and turnover of these structures (Aseervatham, 2020; Wu et al., 2015). Thus, cytoskeletal remodeling represents an active determinant of the cellular plasticity required for invasive and metastatic behavior rather than merely a consequence of increased motility.

Metastatic progression, however, cannot be attributed to alterations in a single cytoskeletal system. Actin filaments, microtubules, and intermediate filaments constitute an interconnected network in which coordinated changes regulate cellular deformability, force generation, intracellular transport, polarity, and structural stability (Lim et al., 2026). Actin remodeling is particularly important for membrane protrusion, cortical organization, and contractility, whereas microtubules contribute to intracellular trafficking and directional organization. Intermediate filaments provide mechanical resilience and preserve cellular integrity under physical stress. Their coordinated reorganization allows tumor cells to adapt their mechanical and structural properties to the changing physical conditions encountered during metastatic dissemination (Lim et al., 2026).

A major regulatory component of cytoskeletal dynamics is the Rho family of small GTPases, including Ras-related C3 botulinum toxin substrate 1 (Rac), Cell division control protein 42 homolog (Cdc42), and Ras homolog family member A (RhoA). These molecules exert complementary effects on actin organisation and coordinate membrane protrusion, cell polarity, and actomyosin-dependent contractility (Lee et al., 2025). Their spatiotemporally regulated activation enables tumour cells to establish front–rear polarity and generate the mechanical forces required for directional and invasive migration (Mishra et al., 2026). Importantly, Rho GTPase signalling is integrated with adhesion-dependent and mechanosensitive pathways, thereby linking cytoskeletal reorganisation to cell–matrix interactions, traction force generation, and the remodelling of adhesive structures during invasion (Aseervatham, 2020).

The importance of cytoskeletal remodeling in cancer extends beyond the mechanical execution of cell movement. Cytoskeletal regulators can integrate structural changes with signaling programs that determine tumor

cell plasticity, invasive capacity, metastatic competence, and adaptation to the tumor microenvironment (Dagar et al., 2026). Dysregulation of proteins involved in actin nucleation, cross-linking, bundling, severing, and membrane attachment has been associated with altered adhesion, protrusion formation, invasion, and metastatic behaviour in several cancer types. For example, dysregulation of the actin-related protein 2/3 complex (Arp2/3) has been linked to invasive phenotypes, whereas proteins such as filamins, α-actinin, and fascin can modulate cell adhesion and migratory capacity. Nevertheless, the effects of individual cytoskeletal regulators are highly context-dependent and may vary according to cellular- and tissue-specific conditions (Li et al., 2022).

Collectively, the cytoskeleton should therefore be considered an integrated regulatory system that connects the structural, mechanical, and signaling properties of malignant cells. Its continuous remodeling provides tumor cells with the plasticity required to sense extracellular conditions, reorganize their architecture, and adopt distinct phenotypic states during the metastatic cascade. Understanding the molecular mechanisms governing cytoskeletal dynamics is consequently important for elucidating cancer progression and metastasis and for identifying cytoskeleton-associated biomarkers and therapeutic targets (Wu et al., 2015). Cytoskeletal remodeling thus represents a mechanistically convergent process through which oncogenic signaling, cell matrix interactions, tissue mechanics, and phenotypic plasticity are integrated to support malignant progression.

In this chapter, the fundamental organization and major components of the cytoskeleton will first be introduced, followed by an examination of actin associated proteins, microtubules, intermediate filaments, and the signaling mechanisms regulating their dynamics in cancer. Particular emphasis will be placed on the contribution of cytoskeletal remodeling to epithelial–mesenchymal plasticity, directed migration, extracellular matrix invasion, adaptation to the tumor microenvironment, and metastatic dissemination. The associated histopathological and structural alterations, as well as the emerging relevance of cytoskeleton-associated molecules as diagnostic and prognostic biomarkers, will subsequently be discussed. Finally, the potential of these molecules as therapeutic targets in cancer will be considered.

## 2. Normal Histology and Embryological Development

### 2.1. Normal Histological Organization of the Cytoskeleton

The cytoskeletal system represents an integrated molecular framework that defines cellular architecture and coordinates the spatial arrangement of intracellular structures. Rather than acting solely as a structural scaffold, the cytoskeleton represents a highly dynamic and integrated system that continuously undergoes spatially and temporally regulated remodeling in response to intracellular programs and extracellular stimuli (Momotyuk et al., 2025). Accordingly, cytoskeletal organization constitutes an essential level of cellular architecture required for the preservation of tissue integrity under physiological conditions. In eukaryotic cells, this organization is generated through the functional interplay of three filament systems with distinct biochemical and mechanical characteristics: actin filaments, microtubules, and intermediate filaments (Peng et al., 2018).

Actin provides a highly adaptable structural network, particularly prominent near the plasma membrane. Assembly of actin subunits generates filamentous structures whose organization is dynamically regulated according to cell type and functional state. These networks support the membrane, maintain intercellular contacts, mediate cell–matrix interactions, support vesicular trafficking, and drive cell division (Lee et al., 2010). A defining characteristic of the actin system is its capacity for continuous turnover, allowing cellular architecture to adjust to changing functional requirements. The formation, stabilization, rearrangement, and disassembly of actin structures are controlled by a broad repertoire of actin associated regulatory proteins, thereby enabling precise spatial regulation of the filament network (Khan & Farhana, 2025).

Microtubules constitute a tubular intracellular system assembled from tubulin subunits and characterized by intrinsic polarity. Their organization provides an internal framework for the positioning and movement of organelles and vesicles, contributes to the establishment of cellular polarity, and becomes particularly important during chromosome segregation and cell division (Wang et al., 2025).

Unlike a permanently fixed intracellular scaffold, the microtubule network cycles through extension and shortening, allowing its architecture to be continuously adjusted. Microtubule-associated factors and molecular motors regulate this behaviour, enabling the network to support both

spatial organisation and intracellular transport. Thus, under physiological conditions, microtubules function as an adaptable intracellular system that coordinates the spatial distribution and directional transport (Lim et al., 2026).

Intermediate filaments differ from the other two filament systems by lacking structural polarity and showing greater mechanical persistence. Their principal contribution is preserving cellular integrity and resisting physical stress. The composition of this filament system varies according to cellular identity and tissue specialization (Ndiaye et al., 2022). Keratin networks characterise epithelial cells, whereas vimentin is prominent in mesenchymal populations; other intermediate filament proteins are enriched in specific differentiated cell types. Through interactions with the plasma membrane and intracellular structures, intermediate filaments help maintain cellular shape and mechanical resilience.

The physiological organization of the cytoskeleton cannot be fully understood by considering the three filament classes independently. Actin filaments, microtubules, and intermediate filaments communicate functionally and cooperate to preserve cellular architecture while permitting controlled structural adaptation. Their coordinated activity helps position intracellular components appropriately and converts extracellular stimuli into cellular responses. Consequently, the organization of the normal cytoskeleton is better understood as an interconnected regulatory network rather than as a simple collection of individual filament populations.

A particularly important aspect of this organization is the close functional association between cytoskeletal elements and cellular adhesion structures. Epithelial cells maintain tissue architecture through specialized contacts with neighboring cells and through attachment to the underlying extracellular matrix. Cadherin-based junctional complexes are physically connected to the actin network, allowing forces generated or experienced at cell–cell interfaces to be distributed through the cytoskeletal system. Desmosomal structures are associated with intermediate filaments and consequently contribute to the mechanical stability of epithelial layers. At cell matrix interfaces, integrin-containing adhesion complexes establish connections between extracellular matrix components and the actin cytoskeleton through linker proteins such as talin, vinculin, and paxillin.

These molecular connections enable extracellular mechanical information to influence intracellular organization and signaling (Lindberg et al., 2017). The relationship between cytoskeletal architecture and adhesion is therefore reciprocal, not unidirectional. E cadherin associated junctional complexes couple to the actin network and help maintain epithelial cohesion, whereas integrin based contacts connect the extracellular matrix to intracellular actin structures. Through these interfaces, cells can both maintain stable attachment and respond to changes in their physical environment. These connections provide an important structural basis for integrating mechanical and biochemical signals within normal tissues (Giretti et al., 2008). Under physiological conditions, the coordinated arrangement of these components balances structural stability, cellular polarity, tissue cohesion, and the capacity for controlled adaptation. During malignant transformation, disruption of this organization can alter adhesion, cellular mechanics, and migratory behavior. Establishing the normal histological framework of the cytoskeleton is therefore essential for interpreting the structural and functional alterations that emerge during cancer progression, particularly those associated with loss of tissue organization, altered cell–matrix interactions, and acquisition of invasive properties (Weber et al., 1982).

### 2.2. Embryological Development of the Cytoskeleton

Embryonic development involves a continuous coordination of cell proliferation, differentiation, repositioning, and tissue assembly. During this period, cells must acquire the ability to modify their morphology, establish spatially and temporally coordinated directional behavior, and adjust their physical relationships with neighboring cells and the surrounding extracellular environment. The cytoskeleton provides an important structural and regulatory basis for these developmental adaptations (Manna et al., 2025). Actin networks, microtubules, and intermediate filaments are dynamically reorganized in accordance with the changing morphogenetic requirements of developing cells, thereby contributing to polarity establishment, morphological plasticity, intracellular organization, and tissue shaping. Cytoskeletal plasticity can therefore be regarded as an important interface between cell-autonomous behaviours and coordinated morphogenetic processes that drive

progressive formation of embryonic tissues (Karr et al., 1986; Lim et al., 2021).

During early embryogenesis, extensive rearrangement of cellular populations is required to establish the emerging body plan. Gastrulation represents one of the major developmental settings in which this type of cellular reorganization occurs. As embryonic cells change their relative positions, they must repeatedly adjust their shape, polarity, adhesion, and mechanical behavior (Leptin, 2005). Remodeling of the actin network contributes to these morphological transitions, while changes in cellular polarity and intercellular contacts help coordinate the direction and extent of cellular rearrangement. Gastrulation therefore provides a physiological example in which dynamic cytoskeletal regulation is directly coupled to large scale tissue morphogenesis (Lv et al., 2021).

The cytoskeleton's contribution becomes particularly evident during developmental processes that require cells to move toward specific anatomical destinations. Directional migration depends on the establishment of a polarized cellular organization, localized changes in membrane architecture, and continuous adaptation of contacts with the surrounding extracellular matrix. Actin based remodeling contributes to the generation of structures required for movement and to the redistribution of cellular mass, whereas microtubule organization supports intracellular orientation and spatial coordination. The interaction between these systems enables developing cells to adjust their behavior as they encounter different extracellular conditions. Developmental epithelial-mesenchymal transition (EMT) is a clear example of how structural remodelling can alter embryonic cell behaviour. In selected developmental contexts, epithelial cells acquire a less constrained and more motile phenotype, allowing them to participate in tissue formation at sites distant from their original position. This transition involves coordinated changes in intercellular organization, cell polarity, and cytoskeletal architecture. Remodeling of actin contributes to changes in cellular morphology and movement, while alterations in intermediate filament composition accompany the acquisition of mesenchymal characteristics. Thus, developmental EMT should be considered as a coordinated change in cellular architecture rather than simply a modification of cell adhesion (Stemmler., 2008).

The structural changes associated with developmental EMT also involve the interfaces between cells and their surrounding environment. Modulation of intercellular junctions facilitates changes in tissue cohesion, while reorganization of cell–matrix contacts alters the mechanical relationship between migrating cells and the extracellular matrix. These events occur together with cytoskeletal remodeling and allow cells to transition from a relatively constrained epithelial organization toward a state that is more compatible with movement and tissue redistribution. The integration of cytoskeletal and adhesion-related changes is therefore an important feature of developmental cellular plasticity. The relevance of these developmental mechanisms extends to cancer biology because malignant cells can reacquire cellular characteristics that resemble those used during embryonic tissue remodeling. During cancer-associated EMT, alterations in polarity, intercellular adhesion, cytoskeletal organisation, and cell motility may collectively promote a more migratory phenotype. This resemblance suggests that tumor cells can exploit cellular programs that normally operate during development to overcome tissue constraints and acquire invasive properties. Importantly, however, EMT in cancer does not necessarily represent a complete and irreversible conversion from an epithelial to a mesenchymal state. Tumor cells may instead occupy intermediate phenotypes and move between different cellular states, emphasizing the importance of cytoskeletal flexibility in malignant progression.

From this perspective, embryonic development provides a physiological framework for understanding how cytoskeletal plasticity can influence cancer cell behavior. Mechanisms that normally coordinate polarity, cellular movement, adhesion, and tissue remodeling during development may be reconfigured in malignant cells to support migration, invasion, and metastatic dissemination. Understanding the developmental regulation of cytoskeletal architecture therefore provides an important conceptual basis for interpreting the structural changes observed during tumor progression and for examining how phenotypic flexibility contributes to the acquisition of metastatic characteristics (Karr et al., 1986; Lim et al., 2021).

## 3. Molecular, Histological, and Cellular Structure and Related Major Signaling Pathways

### 3.1. Molecular and Cellular Organization of the Cytoskeleton

At the molecular level, the cytoskeleton represents a highly interconnected regulatory network in which filamentous polymers, associated proteins, molecular motors, and membrane-associated complexes cooperate to determine cellular architecture and behaviour. Rather than functioning as isolated structural elements, actin filaments, microtubules, and intermediate filaments are continuously regulated through mechanisms controlling polymer assembly, disassembly, stabilisation, cross-linking, and spatial redistribution. This dynamic organization enables the cytoskeleton to respond rapidly to changes in intracellular and extracellular conditions and provides a molecular basis for cellular plasticity (Lim et al., 2026; Fletcher et al., 2010).

The molecular properties of actin are particularly important for rapid cytoskeletal remodeling. The balance between actin polymerisation and depolymerisation determines the formation and turnover of distinct filamentous structures, while actin-associated regulatory proteins control filament nucleation, elongation, severing, capping, cross-linking, and bundling. Through the coordinated regulation of these processes, actin networks can be selectively reorganized within different cellular compartments, thereby generating the structural basis for changes in cell shape, membrane protrusion, adhesion, and motility. Such molecular regulation is particularly relevant to cancer cells because localized actin remodeling can support the formation of protrusive and contractile structures required for directional migration and invasion.

Microtubule organization is similarly dependent on molecular mechanisms that regulate polymer stability and dynamic behavior. Microtubule-associated proteins modulate the assembly and stability of tubulin polymers, whereas motor proteins establish directional interactions between microtubules and intracellular cargo. Consequently, microtubules do not merely provide an intracellular transport system; they also help coordinate cellular polarity and movement. Alterations in microtubule dynamics can therefore influence the distribution of intracellular components and the organization of migratory cells, establishing a

molecular connection between cytoskeletal remodeling and malignant cellular behavior.

Intermediate filaments provide an additional molecular layer of cytoskeletal regulation. Their composition is closely linked to cellular differentiation and phenotypic state, while their interactions with other cytoskeletal components help integrate mechanical and structural information. In particular, changes in keratin- and vimentin-based networks accompany alterations in cellular phenotype. During EMT, increased prominence and reorganization of vimentin are associated with changes in cellular morphology and adhesion, illustrating how alterations in intermediate filament organization can accompany the acquisition of a more migratory phenotype.

An important molecular feature of cytoskeletal organization is the extensive cross talk among different filament systems. Proteins such as plectin, spectrin, ankyrin, and spectraplakins establish physical and functional connections between cytoskeletal components, while filamins and other actin-associated proteins link actin networks with membrane-associated structures and signalling molecules. These interactions distribute mechanical forces and molecular signals across the cell and prevent the cytoskeleton from functioning as a collection of independent filament systems. Instead, they generate an integrated molecular framework capable of coordinating structural remodeling with changes in cellular behavior.

The molecular organisation of the cytoskeleton is further integrated with cell–cell and cell–matrix adhesion complexes. Cadherin–catenin complexes link intercellular adhesion sites to the actin cytoskeleton, whereas integrin-containing focal adhesion structures establish molecular connections between the extracellular matrix and intracellular actin networks. These complexes function as dynamic signalling interfaces rather than passive attachment sites. Mechanical forces generated by actomyosin activity can modify adhesion organisation, while extracellular mechanical and biochemical cues can alter cytoskeletal architecture and cellular responses (Bershadasky et al., 2012). Thus, cytoskeletal remodelling emerges from reciprocal interactions among filament dynamics, adhesion complexes, mechanical forces, and intracellular signalling. This molecular integration is particularly significant in cancer

progression, where alterations in cytoskeletal regulatory proteins and adhesion-associated signalling can shift the balance between cellular stability and plasticity. Changes in actin organisation, microtubule dynamics, intermediate filament composition, and cytoskeleton–adhesion coupling may collectively promote altered cell polarity, enhanced contractility, reduced tissue cohesion, and increased migratory capacity (Vignaud et al., 2012). Importantly, these molecular alterations also provide a mechanistic interface through which extracellular matrix properties and intracellular signalling pathways can regulate cytoskeletal behaviour. Therefore, understanding the molecular organisation and regulatory interactions of the cytoskeleton provides the necessary framework for examining the major signalling pathways that control cytoskeletal remodelling during cancer progression and metastasis.

### 3.2. Related Main Signaling Pathways Regulating Cytoskeletal Dynamics

Cytoskeletal remodeling is regulated by an interconnected signaling network in which growth factor receptors, adhesion molecules, extracellular matrix cues, intracellular kinases, and small GTPases converge to control cytoskeletal organization and cellular behavior (Provenzano et al., 2011). These signaling pathways are extensively interconnected, allowing biochemical and mechanical cues to be integrated and subsequently reflected in cellular polarity, adhesive behavior, contractile activity, and motility.

Rho family GTPases are major regulators in this network, with RhoA, Rac1, and Cdc42 serving as key mediators between extracellular stimuli and actin reorganisation. Rho-associated coiled-coil-containing protein kinase ROCK)-dependent RhoA signalling predominantly enhances actomyosin contractility and stress fibre assembly, whereas Rac1 and Cdc42 contribute to protrusive actin organisation and polarity through regulatory mechanisms involving Wiskott-Aldrich Syndrome Protein/WASP Family Verprolin Homologous Protein WASP/WAVE) proteins and the Arp2/3 complex (Haga et al., 2016; Frugtniet et al., 2015; Guan et al., 2023). Their coordinated and spatially restricted activation therefore establishes the balance between protrusion, contraction, and directional movement that underlies cancer cell migration. These GTPase-

dependent responses are closely connected to Phosphoinositide 3 Kinase/Protein Kinase B (PI3K/AKT) signalling, which links growth factor stimulation to cytoskeletal organisation, adhesion, survival, and motility (Deng et al., 2022). In malignant cells, PI3K/AKT activity can interact with Focal Adhesion Kinase (FAK) and Mitogen-Activated Protein Kinase (MAPK)-associated signalling and cytoskeletal regulators, thereby coupling proliferative and survival signals with increased cellular plasticity and motile behaviour (Lien et al., 2017). In parallel, integrin engagement with the extracellular matrix activates the FAK/Proto-oncogene tyrosine protein kinase Src (Src) axis at focal adhesions, providing a mechanochemical interface between extracellular matrix properties, actin organisation, and intracellular signalling. Crosstalk among FAK/Src, Rho family GTPases, and PI3K/AKT enables adhesion maturation and cytoskeletal force generation to be dynamically coordinated during migration (Bershadasky et al., 2012).

MAPK signalling, including Extracellular Signal-Regulated Kinase ERK), Jun N-Terminal Kinase (JNK), and p38, further integrates growth- and stress-associated stimuli with cytoskeletal and adhesion-associated responses. Through phosphorylation-dependent regulation of relevant cellular components, these pathways can influence cell morphology, proliferation, and migration (Kciuk et al., 2022). Thus, PI3K/AKT, FAK/Src, Rho GTPases, and MAPK signalling form an interconnected regulatory framework through which extracellular stimuli are translated into structural and behavioural changes.

Transforming Growth Factor-β (TGF-β) signalling further links cytoskeletal alterations with the acquisition of phenotypic plasticity. Through its effects on RhoA-mediated actomyosin organisation, intercellular adhesion, and stress fibre assembly, TGF-β can induce cytoskeletal changes that favour EMT-associated remodelling and enhanced cellular motility (Moujaber et al., 2020; Hou et al., 2025). These structural alterations also intersect with Wnt/β-catenin signaling, as β-catenin serves both as a component of cell–cell adhesion complexes and as a regulator of transcriptional activity. Accordingly, remodelling of cytoskeletal and junctional structures may be linked to sustained

alterations in transcriptional activity, proliferative behaviour, and EMT-related cellular characteristics (Liu et al., 2022).

Cytoskeleton-derived tension and extracellular matrix mechanical properties can also modulate the Hippo pathway effectors Yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ). Matrix rigidity, cell density, and actin-generated tension influence YAP/TAZ activity and subcellular distribution, providing a mechanism by which mechanical conditions affect transcriptional programs governing proliferation and cellular plasticity (Guan et al., 2023). Notch and Hedgehog signalling further intersect with these networks by modulating cell communication, differentiation, and tissue organisation, contributing to the coordinated regulation of cytoskeletal behaviour, EMT, and invasive phenotypes in cancer (Lian et al., 2021). Collectively, these pathways should therefore be considered components of a highly interconnected signaling network rather than independent regulatory cascades. Their coordinated activity enables cancer cells to integrate growth, adhesion, mechanical, and developmental signals and convert them into cytoskeletal remodeling, phenotypic plasticity, migration, and ultimately invasive behavior.

## 4. Cytoskeletal Remodeling During Epithelial–Mesenchymal Transition (EMT)

EMT is a dynamic cellular program through which epithelial cells progressively reduce their dependence on tissue architecture and acquire properties that favor motility and adaptation to changing environmental conditions. Although this program can occur transiently during physiological processes such as embryonic development and tissue repair, its inappropriate or sustained activation in tumors is closely associated with invasive behavior and metastatic progression (Huang et al., 2022). The phenotypic consequences of EMT cannot be attributed solely to transcriptional changes. A parallel reorganization of the cytoskeletal machinery is required to physically support the transition toward a more motile and mechanically adaptable cellular state.

In epithelial tissues, cytoskeletal organization is closely integrated with intercellular junctions and attachment to the basement membrane. As EMT progresses, this structural arrangement is progressively modified.

Weakening of adherens junctions and changes in desmosomal components reduce the mechanical continuity between neighboring cells, while alterations in polarity facilitate the establishment of a cell architecture more compatible with directional movement. A prominent molecular event accompanying this transition is reduced E-cadherin. Loss of E-cadherin-mediated adhesion modifies the connection between cortical actin and cell–cell junctions, thereby facilitating cellular separation and independent movement. At the transcriptional level, EMT-associated regulators, including Snail, Slug, ZEB, and TWIST, repress epithelial programs and drive the acquisition of mesenchymal characteristics (Datta et al., 2021). The resulting disruption of junctional–cytoskeletal coupling not only weakens epithelial cohesion but also alters the spatial distribution of cortical forces, thereby facilitating the transition toward a more dynamically organized and mechanically adaptable cellular phenotype.

The loss of adhesion and cytoskeletal reorganization are mechanistically interconnected during EMT. Disrupting E-cadherin-based contacts changes the spatial distribution of the actin network and permits the formation of alternative cytoskeletal arrangements at the cell periphery. At the same time, β-catenin can shift from its structural role at cell junctions toward signaling functions associated with nuclear gene regulation. Consequently, alterations occurring at the plasma membrane can become coupled to transcriptional programs that maintain the mesenchymal phenotype. This reciprocal relationship allows EMT to produce not only physical separation between cells but also a broader remodeling of cellular morphology and behavior (Datta et al., 2021).

Intermediate filament organisation undergoes a characteristic transition during EMT. The keratin-rich network associated with epithelial identity becomes less prominent, whereas vimentin-containing structures become increasingly evident in mesenchymal cells (Infante & Etienne-Manneville, 2022). Vimentin contributes to the cell's mechanical adaptability and interacts functionally with actin and microtubule systems. Through these interactions, it can influence cell morphology, intracellular organisation, and the structural requirements of directional movement. Accordingly, elevated vimentin expression may reflect not only the phenotypic progression of EMT but also the functional reorganisation of the cytoskeletal architecture that supports migratory and invasive behaviour.

Microtubules also undergo functional remodelling during EMT. Alterations in microtubule stability and post-translational modifications can influence cellular motility and the expression of EMT-associated programmes. Changes in tubulin acetylation or detyrosination, for instance, have been linked to mechanisms involving cell adhesion, AKT signalling, and Rho family GTPases. In addition, EMT regulators such as Snail and Twist have been associated with modifications of tubulin properties that favour invasive and migratory behaviour. Collectively, these findings indicate that microtubules have an active regulatory role during EMT beyond their conventional structural function. Among the major cytoskeletal components, actin reorganisation is particularly prominent in producing the morphological changes associated with EMT. The relatively organised cortical actin arrangement characteristic of epithelial cells can be replaced by more prominent stress fibres, protrusive structures, and actomyosin-based contractile assemblies. F-actin structures associated with focal adhesions transmit force to the extracellular matrix, while newly formed peripheral actin networks promote plasma membrane extension. Consequently, specialised actin-rich structures such as lamellipodia, filopodia, and invadopodia may develop to support environmental sensing, extracellular matrix interaction, and invasive movement (Haynes et al., 2011).

The spatial arrangement of these actin based structures is governed by a complex regulatory network. Branched actin networks are generated in part through WASP/WAVE dependent activation of the Arp2/3 complex, while other actin associated proteins control filament bundling, severing, stabilization, and spatial rearrangement. Myosin II generates contractile forces through its adenosine triphosphate (ATP)-dependent motor activity and thereby contributes to the mechanical behaviour of the remodelled cell. Consequently, actin remodelling during EMT represents not simply a change in filament abundance but a comprehensive alteration in filament architecture, localisation, connectivity, and force-generating capacity (Frugtniet et al., 2015).

The cytoskeletal consequences of EMT are further influenced by the mechanical environment surrounding the cell. Increased extracellular matrix stiffness and actomyosin-generated tension can transmit mechanical information to the nucleus through regulators such as YAP and

TAZ. In parallel, pathways including TGF-β and Wnt/β-catenin can sustain EMT-associated transcriptional programs and reinforce changes in adhesion and cytoskeletal organisation. These mechanisms establish reciprocal communication among mechanical inputs, cell-surface adhesion systems, and nuclear gene regulation, allowing the cell to continuously adjust its phenotype to its environment (Gonzalez et al., 2014).

EMT should therefore be regarded as a coordinated remodeling state rather than as a simple loss of epithelial characteristics. Actin networks provide the structural basis for force production and protrusion, microtubules contribute to polarity and intracellular organization, and intermediate filaments enhance mechanical adaptability. The functional cooperation of these three systems enables EMT-associated cancer cells to reorganise their architecture while responding to different physical and biochemical conditions encountered during invasion and metastasis.

## 5. Cytoskeletal Remodeling During Cell Migration

Cell migration constitutes a mechanically regulated process in which the cytoskeletal machinery undergoes continuous reorganization according to spatial and temporal requirements. In metastatic cancer cells, physiological mechanisms of migration are repurposed to support tumor cell dissemination, tissue invasion, vascular entry, and movement toward distant sites (Li et al., 2025). Successful movement requires cells to interpret chemical and mechanical information from their surroundings, establish polarity, generate actin-dependent protrusions, form transient matrix contacts, and coordinate contractile forces. Migration therefore represents a repetitive cycle of protrusion, attachment, force generation, and detachment rather than a single isolated cellular event. Directional movement depends on the emergence of functionally distinct front and rear regions. Actin polymerization at the leading edge drives membrane extension, while contractile activity at the rear contributes to forward displacement of the cell body. This spatial organization creates a polarized mechanical system in which protrusive and contractile activities occur in different cellular compartments. Maintenance of this polarity involves localised activation of Rac1 and Cdc42 toward the front and RhoA-dependent contractile signalling toward the rear (Kalli et al., 2011).

The formation of actin-driven membrane protrusions is an early structural step in migration. Lamellipodia are generated by densely branched actin arrays that extend the leading membrane, whereas filopodia consist predominantly of bundled actin filaments and project beyond the cell boundary to probe the surrounding environment. Rac1-dependent WAVE–Arp2/3 signalling preferentially supports branched actin formation, whereas Cdc42, together with WASP-related mechanisms, promotes filopodial organisation. These protrusions therefore serve complementary functions: one provides a broad platform for forward movement, while the other assists environmental exploration and directional sensing (Liu et al., 2020).

Protrusion alone is insufficient for productive migration. Newly extended regions of the cell must establish transient connections with the extracellular matrix so that internally generated forces can be transmitted to the substrate. Integrins and focal adhesion-associated proteins provide this mechanical linkage. The organization and regulation of these adhesion complexes involve talin, vinculin, paxillin, kindlin, and FAK, which collectively establish a molecular link between actin networks and the extracellular matrix. During migration, adhesions formed near the advancing front support forward movement, whereas older contacts toward the rear must be disassembled to permit cellular translocation. Effective cell migration therefore requires dynamic assembly and disassembly of adhesion sites rather than stable and continuous substrate attachment.

Focal adhesion dynamics are closely coupled to cytoskeletal force production. Tension generated by the actomyosin machinery can influence the maturation and mechanical properties of adhesion complexes, while matrix stiffness and composition can modify actin organization and cellular contractility. The integrin–FAK/Src pathway serves as a key mechanochemical interface linking extracellular mechanical cues to intracellular cytoskeletal responses. Interactions with Rho family GTPases and PI3K/AKT additionally coordinate protrusive activity, adhesion dynamics, and contractile behavior in response to the mechanical properties of the surrounding matrix.

Contractile activity at the rear of the cell is equally important for completion of the migratory cycle. Interaction between actin filaments and

myosin II produces forces that facilitate cell body movement and release rearward adhesions. RhoA–ROCK signaling is an important regulator of this contractile state. In contrast, localized Rac1 and Cdc42 activity toward the leading region promotes continued actin assembly and membrane protrusion. The simultaneous generation of new structures at the front and removal of existing structures at the rear therefore forms a coordinated cycle that drives directional locomotion (Tsai et al., 2015).

Cancer cells can employ more than one mode of locomotion. Depending on matrix density, stiffness, geometry, and other properties of the tumor microenvironment, they may adopt different migratory strategies. Mesenchymal migration is characterised by prominent protrusive activity and integrin-mediated adhesion, whereas amoeboid movement may be favoured under conditions that support greater actomyosin-driven contractility. The cytoskeletal network's plasticity enables transitions between these states, particularly through changes in actin architecture and myosin II activity. This flexibility allows metastatic cells to adjust their mode of movement when encountering different tissue barriers and extracellular matrix configurations. Microtubules and intermediate filaments also contribute to this migratory machinery. Microtubules help maintain cell polarity, support intracellular trafficking, and coordinate the forces required for protrusion. Intermediate filaments, particularly vimentin, can influence cellular mechanics and focal adhesion organization and thereby affect migratory capacity. Thus, metastatic cell motility reflects the coordinated contribution of actin, microtubule, and intermediate filament networks rather than regulation by the actin system alone (Datta et al., 2021).

Overall, cell migration is characterized by continuous cytoskeletal adaptation to the surrounding environment. Formation of actin-rich protrusions at the front, establishment of matrix adhesions, generation of actomyosin-dependent forces, and release of rearward contacts constitute interconnected stages of the same migratory cycle. In cancer cells, the ability to rapidly reorganise these structures provides a major source of phenotypic plasticity and enables adaptation to heterogeneous tissue environments. Live-cell imaging studies showing rapid changes in actin networks, focal adhesions, and myosin II further support the idea that

cytoskeletal plasticity is a fundamental determinant of metastatic cell movement (DeWane et al., 2021).

## 6. Cytoskeletal Remodeling During Invasion

Invasion is a multifaceted cellular process that enables malignant cells to leave the primary tumor and infiltrate adjacent tissues. Invasive progression requires more than cellular displacement; it involves adaptation to extracellular mechanical conditions, dynamic cell–matrix interactions, local matrix remodeling, and generation of sufficient force to overcome tissue constraints. Cytoskeletal remodeling is therefore closely integrated with the invasive phenotype, because changes in filament organization determine both the morphology of the tumor cell and its capacity to interact with the extracellular matrix. In malignant cells, these adaptations are reflected by the formation and redistribution of specialized membrane structures, including lamellipodia, filopodia and invadopodia.

Invadopodia are among the characteristic cytoskeletal structures associated with malignant invasion. These structures extend beyond a simple protrusive function by serving as localized sites where actin organization, adhesion, and extracellular matrix remodeling are spatially coordinated. Their organisation generally involves a central region enriched in polymerised actin together with surrounding adhesion-associated components. Molecular regulators such as WASP/Neural Wiskott-Aldrich Syndrome Protein N-WASP), Arp2/3, cortactin, Cdc42, and Src contribute to the establishment and maturation of these structures. Through this coordinated organization, invasive cells can simultaneously generate localized mechanical activity and position molecular machinery required for interaction with the extracellular matrix (Xiang et al., 2017).

Formation of invadopodia enables localised remodelling of the extracellular matrix at sites of tumour cell invasion. Proteolytic components delivered to these specialised cellular domains facilitate degradation of matrix substrates at sites where the tumour cell is attempting to advance. Membrane Type 1 Matrix Metalloproteinase/Matrix Metalloproteinase 14 MT1-MMP/MMP-14) and Matrix Metalloproteinase 2 MMP-2) contribute to this process, as their proteolytic activity degrades matrix components and reduces the structural barriers invading cells encounter. Therefore, cytoskeletal

remodelling contributes not only to the morphological organisation of invasive cells but also to the spatial organisation required for focal extracellular matrix degradation.

The invasive phenotype additionally depends on cooperation between actin and microtubule systems. Reorganization of actin provides the structural basis for membrane extension and force production, whereas microtubules contribute to intracellular trafficking and the delivery of selected cargos toward specialized cellular domains. Changes in microtubule organization within protrusive regions further indicate that invasion is supported by coordinated activity of multiple cytoskeletal systems rather than by actin alone. Signalling mechanisms involving Hepatocyte Growth Factor (HGF), Rac1, Activated Kinase 1 (PAK1), and stathmin provide an additional link between extracellular stimulation, microtubule behaviour, and the migratory capacity of tumour cells (Egawa et al., 2006).

Spatial control of Rho family GTPases provides another major layer of regulation. RhoA, Rac1, and Cdc42 produce distinct cytoskeletal outputs through spatially restricted activation, generating actin architectures suited to different phases of invasion. RhoA primarily supports contractile actomyosin organization, while Rac1 promotes protrusive remodeling and Cdc42 contributes to filopodial assembly. The coordinated distribution of these activities allows cancer cells to establish distinct functional regions and continuously modify their morphology while progressing through the surrounding matrix (Aseervatham., 2020).

Tumor cells may also alter their mode of invasion according to the characteristics of the surrounding stroma. A mesenchymal strategy generally involves elongated morphology, pronounced cell–matrix interactions, focal adhesion activity and greater dependence on extracellular proteolysis. In contrast, amoeboid behavior is characterized by a more rounded morphology and relies strongly on actomyosin generated forces and RhoA–ROCK signaling. This capacity for phenotypic switching reflects the plasticity of the cancer cytoskeleton and may enable tumor cells to preserve invasive competence when a specific migratory route becomes unfavorable or inhibited (Yılmaz et al., 2009).

Invasion can further occur through collective rather than completely individual cell movement. In this setting, a subset of cells at the advancing

front modifies the surrounding matrix, whereas neighbouring cells maintain intercellular contacts and follow the remodelled path. Such behavior indicates that complete disruption of epithelial adhesion is not an obligatory requirement for invasive progression. Changes in E-cadherin-associated complexes can simultaneously affect cell–cell adhesion, β-catenin signalling, and cytoskeletal organisation, linking alterations in tissue architecture with the acquisition of invasive properties.

## 7. Mechanotransduction and Cytoskeletal Remodeling

A comprehensive understanding of cancer progression requires attention to oncogenic and genetic alterations as well as the physical conditions surrounding malignant cells. Tumour cell behaviour is strongly affected by the composition, structural organisation, and mechanical characteristics of the extracellular matrix. Changes in matrix organisation modify the mechanical inputs perceived by the cell and consequently affect the organisation of the cytoskeletal system. Mechanotransduction encompasses the molecular processes by which physical forces acting on cells are sensed and converted into intracellular biochemical signalling events. Within this framework, the cytoskeleton acts as a force-bearing network that distributes and transmits mechanical loads while coordinating cellular adaptation to changes in the physical environment (Silver et al., 2026).

Tumour-associated remodelling of the extracellular matrix can alter its mechanical properties through processes such as increased collagen deposition, modification of matrix architecture and activation of stromal cells. A mechanically altered matrix can promote changes in cell-matrix adhesion and actomyosin organisation, enabling tumour cells to generate and withstand greater contractile forces. Importantly, the relationship between matrix mechanics and the cytoskeleton is reciprocal. The extracellular environment imposes mechanical constraints on the cell, whereas forces produced by the contractile cytoskeleton can further modify the surrounding matrix. This reciprocal interaction may establish a reinforcing mechanical circuit that favours malignant progression (Martino et al., 2018).

Integrin-based adhesion complexes constitute a principal cellular interface for sensing mechanical cues originating from the extracellular

matrix. Binding of integrins to matrix components facilitates recruitment and organization of proteins such as talin, vinculin, kindlin, paxillin and FAK, thereby establishing a physical connection between the ECM and the actin cytoskeleton. These complexes are not structurally static. Their molecular composition and stability can change in response to mechanical loading. For example, mechanical loading of talin can induce conformational changes that expose vinculin-interacting sites and consequently reinforce adhesion complex stability. Such molecular responses illustrate how physical force can be converted into a biochemical signal at the cell–matrix interface (Martino et al., 2018).

Signals generated at focal adhesions can subsequently influence FAK/Src activity, Rho family GTPases, and PI3K/AKT signalling. By converging on cytoskeletal regulatory mechanisms, these pathways adjust actin architecture, cellular contractility, and focal adhesion turnover in response to matrix mechanics. RhoA–ROCK activation increases actomyosin-generated tension and can strengthen the traction forces produced by tumour cells against mechanically stiff substrates. Consequently, matrix mechanics and intracellular contractility become connected through a continuous feedback mechanism. Mechanical cues can also be transmitted from the cytoplasmic cytoskeleton to the nucleus. Actin-generated forces may reach the nuclear envelope through physical coupling mechanisms involving the Linker of Nucleoskeleton and Cytoskeleton LINC) complex. Changes in mechanical loading may therefore influence nuclear morphology and the organisation of chromatin, providing a mechanism through which cytoskeletal alterations can produce longer-lasting effects on gene regulation. This physical continuity between the cytoskeleton and nucleus links cellular mechanics with phenotypic adaptation (Datta et al., 2021).

The Hippo–YAP/TAZ axis is a major signalling pathway that converts mechanical cues into changes in transcriptional activity. Under low mechanical tension, YAP and TAZ remain predominantly cytoplasmic and exhibit reduced transcriptional activity. Conversely, increased matrix stiffness, elevated actomyosin tension, and enhanced cell–matrix engagement promote nuclear accumulation of YAP/TAZ. Once in the nucleus, YAP/TAZ interact with TEA Domain Transcription Factor (TEAD) transcription factors to regulate gene expression programmes

involved in proliferation, cellular plasticity, adhesion, and extracellular matrix organisation. Consequently, alterations in the mechanical microenvironment can influence gene expression through a pathway that links extracellular mechanics to cytoskeletal organisation. Mechanical signalling may become particularly persistent within the tumour microenvironment, as tumour and stromal cells continuously modify one another's physical surroundings. Cancer-associated fibroblasts and other stromal populations can alter matrix organisation and rigidity, while increased contractile activity of tumour cells contributes to further matrix remodelling. This reciprocal interaction can maintain activation of mechanosensitive networks involving integrins, FAK/Src, Rho GTPases, and YAP/TAZ. Sustained signalling through these pathways may consequently support not only tumour growth but also migration, tissue invasion, and metastatic adaptation (Dorland et al., 2017; Farge., 2011).

Mechanotransduction and cytoskeletal remodeling should therefore be regarded as interconnected components of a single adaptive system. The physical properties of the extracellular matrix continuously influence cytoskeletal architecture, while forces generated by the cytoskeleton feed back to alter cell–matrix interactions and the surrounding tissue. This bidirectional relationship provides cancer cells with the capacity to adjust their morphology, adhesion, contractility and gene expression programs according to changing mechanical conditions, thereby facilitating persistent invasive and metastatic behavior.

## 8. Clinical Manifestations and Diagnostic Biomarkers

Alterations in cellular architecture accompanying malignant transformation are not limited to changes in tumor cell behavior; they may also generate molecular features with potential diagnostic and prognostic relevance. Variations in the abundance and distribution of cytoskeletal proteins can reflect fundamental characteristics of malignant cells, including altered morphology, reduced intercellular adhesion, enhanced motility, and invasive capacity (Mierke et al., 2025). Accordingly, analysis of cytoskeletal components within tumor tissues may provide complementary information regarding the biological phenotype and progression of cancer. Comparative proteomic investigations have demonstrated that several cytoskeletal proteins exhibit differential

abundance between normal tissues, primary tumors, and metastatic lesions. These observations suggest that cytoskeletal proteins may provide molecular information about tumor phenotype, disease progression, and metastatic potential in addition to their established structural functions (Das et al., 2023).

Among the cytoskeletal markers investigated in cancer, vimentin and E-cadherin are particularly relevant because of their association with EMT. Increased vimentin expression is commonly associated with a more adaptable and motile cellular phenotype, whereas reduced E-cadherin expression reflects weakened epithelial cell–cell interactions. Joint assessment of vimentin and E-cadherin may consequently help characterise the degree of phenotypic transition from an epithelial state towards a more invasive cellular phenotype (Pandita et al., 2021). Nevertheless, interpreting a single cytoskeletal protein may be insufficient, as the biological significance of altered protein abundance can vary by tumour origin and disease context. A combined assessment of several cytoskeletal components and their intracellular localisation may consequently provide a more informative molecular profile.

β-catenin represents another important molecular link between cytoskeletal organization and malignant progression. In addition to its contribution to cell–cell adhesion complexes, β-catenin participates in intracellular signaling and can change its functional role according to its cellular localization. Accordingly, evaluating the intracellular distribution of β-catenin may be more informative than considering its overall abundance alone when assessing tumor cell alterations. Similarly, proteins associated with focal adhesions and actin organisation can reflect changes in cellular motility and invasive behaviour. Proteomic studies have identified differential expression of several actin-associated proteins, including fascin, filamin, α-actinin, plectin, and desmoplakin, across different malignancies. Altered expression of selected proteins within this group has been linked to differences in tumor cell motility, invasive behavior, and metastatic capacity (Liu et al., 2022).

The clinical relevance of cytoskeletal alterations can also be considered in relation to tumor morphology. Conventional histopathological examination provides information about cellular architecture, polarity, shape, and tissue organization, all of which may change during malignant

progression. Because these phenotypic characteristics are closely connected to the underlying cytoskeletal composition, morphological assessment and molecular profiling can provide complementary perspectives on tumor behavior. Consequently, cytoskeletal phenotyping may contribute not only to tumor classification but also to the estimation of progression and metastatic potential (Li et al., 2021). Another area of interest is investigating cytoskeletal associated molecules in minimally invasive clinical samples. Circulating tumor cells, extracellular vesicles, and tumor derived molecular material may provide an opportunity to monitor changes originating from the primary tumor at the systemic level. In particular, simultaneous assessment of epithelial and mesenchymal characteristics in circulating tumor cells may help characterize their metastatic phenotype. Researchers are also investigating cytoskeleton-related proteins and nucleic acids transported by extracellular vesicles as potential indicators for monitoring disease status and therapeutic response (Spada et al., 2021).

Despite these advances, cytoskeletal proteins should not be regarded as universally applicable biomarkers without consideration of tumor type and biological context. The direction and magnitude of changes in individual proteins may differ between tumor entities and may also vary between primary and metastatic lesions. Therefore, their clinical interpretation requires integration with tumor histology, disease stage, tissue origin, and complementary molecular markers.

## 9. Experimental Models for Investigating Cytoskeletal Remodeling in Cancer

Investigating cytoskeletal remodelling in cancer requires experimental approaches that span multiple biological scales, from intracellular molecular events to tissue-level organisation and whole-organism behaviour. This requirement arises from the highly context-dependent nature of the cytoskeleton, whose organisation is influenced not only by intracellular regulatory proteins but also by extracellular matrix composition, mechanical properties, cell–cell interactions, and signals originating from the tumour microenvironment. Accordingly, complementary experimental systems have been developed to characterise the organisation of actin filaments, microtubules, and intermediate

filaments and to determine how their dynamic interplay contributes to tumour cell motility, invasion, and metastatic progression (Peng et al., 2018). Accordingly, experimental model selection is crucial because cytoskeletal structure and mechanics depend heavily on cellular context and the physical conditions of the surrounding environment.

Two-dimensional cell culture remains an important experimental framework for dissecting the molecular and cellular mechanisms underlying cytoskeletal remodelling. Growth on planar substrates facilitates the controlled assessment of actin distribution, stress fibre organisation, focal adhesion dynamics, and microtubule architecture. Fluorescence-based approaches, including immunofluorescence microscopy and live-cell imaging, can be employed to monitor temporal changes in cytoskeletal organisation and to characterise the redistribution of cytoskeletal structures during cellular movement. Functional assays such as scratch-wound and Transwell migration assays further enable cytoskeletal alterations to be correlated with migratory behaviour. Despite these advantages, conventional monolayer cultures provide only a simplified representation of the tumour environment because they do not adequately reproduce three-dimensional tissue architecture, matrix heterogeneity, or the mechanical constraints encountered by tumour cells *in vivo* (Li et al., 2026).

Three dimensional culture systems and organoid models provide a more physiologically relevant framework in which cytoskeletal organization can be examined within a spatially complex environment. Cellular morphology, actin distribution, microtubule arrangement, and membrane protrusion formation may differ substantially between three dimensional matrices and conventional monolayer cultures. This distinction is particularly relevant during EMT, when changes in the organization of actin and microtubule networks contribute to the development of invasive cellular phenotypes. Organoid models allow the investigation of cell polarity, collective movement, invasion, and tumor–matrix interactions under conditions that more closely resemble tissue architecture. Moreover, controlled modification of matrix composition and mechanical properties provides an experimental means of examining how the cytoskeleton adapts to changes in the surrounding microenvironment. Patient-derived organoids additionally offer an opportunity to investigate intertumoral

heterogeneity and differential therapeutic responses within individualised experimental systems (Aseervatham, 2020; Caino et al., 2013).

Microfluidic and organ-on-a-chip systems offer complementary platforms for examining how cytoskeletal organisation responds to the tumour microenvironment's physical properties. These systems can reproduce defined flow conditions, spatial confinement, matrix composition, and mechanical constraints, thereby allowing selected features of the metastatic microenvironment to be experimentally reconstructed. Under confined conditions, tumor cells may undergo rapid alterations in actin organization, microtubule architecture, actomyosin contractility, and nuclear morphology as they adapt to restricted spaces. Such models are therefore particularly valuable for examining how cytoskeletal plasticity enables tumor cells to respond to mechanical rather than exclusively biochemical stimuli.

*In vivo* models provide an essential complementary level of analysis by determining whether mechanisms identified in simplified experimental systems remain functionally relevant during tumour development and metastatic dissemination. Xenograft, orthotopic, and genetically engineered animal models can be used to investigate cytoskeletal alterations associated with tumour growth, local invasion, vascular dissemination, and colonisation of distant tissues. Intravital imaging has further enabled direct visualisation of tumour cell behaviour within living tissues, revealing the dynamic reorganisation of actin-rich structures, cellular protrusions, and adhesion sites during metastatic progression. These models are also important for establishing the functional contribution of cytoskeletal regulators to tumour progression and for evaluating whether pharmacological interference with cytoskeletal pathways can modify metastatic behaviour (Coughlin et al., 2013).

Taken together, the experimental system chosen for a cytoskeletal remodelling study substantially shapes the biological interpretation of the resulting observations. Two-dimensional cultures are particularly useful for isolating molecular mechanisms under controlled conditions, whereas three-dimensional organoids provide a more representative tissue context. Microfluidic systems permit systematic manipulation of physical and mechanical constraints, while *in vivo* models establish the relevance of these mechanisms within the complex setting of tumour progression and

metastasis. Integrating these complementary approaches therefore provides a more comprehensive framework for defining how cytoskeletal plasticity is established and how it contributes to the acquisition and maintenance of metastatic phenotypes.

## 10. Current Therapeutic Approaches Targeting Cytoskeletal Remodeling

Growing knowledge of cytoskeletal remodeling in cancer has shifted therapeutic interest toward strategies capable of limiting tumor cell migration, invasion, and metastatic spread in addition to controlling proliferation. Given the essential physiological functions of cytoskeletal networks, current approaches increasingly prioritize aberrantly regulated molecular components and signaling pathways in cancer cells instead of broadly disrupting the cytoskeletal machinery (Deng et al., 2022). This therapeutic shift reflects the recognition that selective interference with cytoskeletal plasticity may provide an opportunity to attenuate malignant cell behavior while minimizing the systemic toxicity associated with direct disruption of essential cytoskeletal structures.

Among cytoskeleton-targeting therapies, microtubule-directed drugs remain the most established clinical agents. Vinca alkaloids interfere with tubulin polymerisation, whereas taxanes favour microtubule stabilisation; both mechanisms can disrupt mitotic progression and affect cellular polarity, motility, and invasion. The clinical activity of these agents is therefore closely related to their ability to interfere with the dynamic instability of microtubules and, consequently, with processes required for cell division and cellular organization. Nevertheless, treatment efficacy is frequently limited by neurotoxicity, drug resistance, and adverse effects on rapidly proliferating normal tissues (Steinmetz et al., 2018). These limitations illustrate the central therapeutic challenge of targeting cytoskeletal structures: the same molecular machinery that supports malignant progression is also indispensable for the physiological function of normal cells.

Another major strategy focuses on the Rho/ROCK signaling pathway, which regulates actomyosin contractility and cytoskeletal organization. Pharmacological inhibition of ROCK decreases myosin light chain phosphorylation and stress fibre organisation and can consequently

attenuate amoeboid migration, a motility phenotype frequently implicated in metastasis. Although compounds such as Fasudil and Y-27632 have shown encouraging anti-metastatic activity in experimental studies, compensatory migration mechanisms and ROCK's physiological functions remain important challenges for clinical translation (Tönges et al., 2011). FAK has emerged as another therapeutic target of interest because it serves as an important signaling link between extracellular matrix cues and intracellular cytoskeletal regulation. Blocking FAK signaling disrupts focal adhesion turnover, attenuates migratory and invasive capacities, and may improve the efficacy of immune checkpoint inhibitors by modifying the tumor microenvironment. Consequently, researchers are currently evaluating FAK inhibitors mainly in combination treatment strategies (Chuang et al., 2022).

Integrins' dual involvement in adhesion and mechanotransduction makes them potential therapeutic targets. Inhibiting integrin subtypes, particularly αvβ3, αvβ5, and β1 integrins, disrupts cell–ECM interactions and reduces invasive potential. However, the functional redundancy among integrin family members has limited the clinical benefit of integrin-targeted monotherapies, supporting their evaluation within combination regimens (Pang et al., 2023).

Direct disruption of actin filaments has demonstrated anti-invasive effects, but the essential role of actin in normal tissues has restricted the clinical application of conventional actin-targeting compounds because of systemic toxicity. Consequently, recent approaches have increasingly investigated regulatory components of the actin machinery including Arp2/3, formins, fascin, cofilin, LIM Domain Kinase (LIMK), tropomyosin, and myosin II as more selective targets for restricting metastatic behavior while potentially limiting systemic toxicity (Lim et al., 2026). Beyond cytoskeletal structural regulators, mechanotransduction pathways have emerged as attractive therapeutic targets. Targeting YAP/TAZ–TEAD signaling can interfere with transcriptional programs that support EMT, invasive behavior, metastatic dissemination, and resistance to therapy. Although preclinical findings are promising, further optimization is required to develop selective inhibitors that preserve the physiological functions of YAP/TAZ in normal tissues (Han et al., 2024).

## 11. Future Perspectives

Continuous advances in cancer biology have highlighted cytoskeletal remodeling as a critical determinant of tumor progression, metastatic dissemination, and therapeutic resistance. The combined application of emerging approaches, including single-cell multi-omics, spatial transcriptomics, high-content live-cell imaging, and AI-assisted computational methods, is expanding the ability to characterise cytoskeletal organisation and its dynamic changes across heterogeneous tumour populations with increasing spatial and temporal precision. Together, these methodologies may provide deeper insight into tumor evolution, phenotypic plasticity, and the multifaceted interactions between malignant cells and their surrounding microenvironment. Importantly, integrating molecular, spatial, and biomechanical information may help distinguish transient cytoskeletal states from stable phenotypic programs associated with metastatic competence.

Future investigations are expected to focus on translating knowledge of cytoskeletal signalling into precision oncology. Cytoskeletal regulatory components, such as Rho GTPases, FAK, the YAP/TAZ axis, and actin-associated proteins, may represent context-dependent therapeutic targets and could be incorporated into rational combinations with immunotherapeutic, targeted, or conventional cytotoxic approaches. However, the therapeutic value of these targets is likely to depend on tumour lineage, molecular context, cytoskeletal dependency, and the characteristics of the surrounding microenvironment. In parallel, patient-derived organoids, organ-on-a-chip platforms, and liquid biopsy technologies are likely to facilitate individualised therapeutic selection, earlier identification of metastatic progression, and more accurate prognostic evaluation. Collectively, these developments support the view that modulation of cytoskeletal remodelling will play an increasingly important role in translational cancer research and personalised therapeutic approaches (Han et al., 2025).

## 12. Conclusions

Cytoskeletal remodeling constitutes a fundamental biological mechanism that contributes to multiple stages of tumor progression and metastatic dissemination. The dynamic network of actin filaments,

microtubules, and intermediate filaments coordinates essential cellular functions, including polarity, adhesion, motility, invasion, and responses to mechanical cues. Deregulation of these processes during malignant transformation enhances tumor cell plasticity, permits switching between distinct migratory behaviors, and facilitates adaptation to changing microenvironmental conditions, ultimately supporting metastatic spread. The coordinated regulation of these processes involves multiple interconnected signaling systems, notably Rho family GTPases, PI3K/AKT, integrin FAK/Src, TGF-β, Wnt/β-catenin, Hippo–YAP/TAZ, Notch, and Hedgehog pathways.

Recent advances in experimental model systems and high-resolution molecular technologies have clearly demonstrated that the cytoskeleton is far more than a structural component of the cell. Instead, it functions as a dynamic regulatory interface that integrates biochemical and mechanical cues, thereby coordinating cellular responses to the surrounding environment. Consequently, cytoskeleton-associated molecules have emerged as valuable prognostic biomarkers and promising therapeutic targets in cancer. Accordingly, therapeutic approaches targeting cytoskeletal plasticity in metastatic disease may complement conventional antiproliferative treatments. Nevertheless, given the indispensable roles of the cytoskeleton in normal cellular physiology, developing highly selective and safe therapeutic approaches remains a major challenge for future research. Further clarification of the molecular and biomechanical basis of cytoskeletal remodelling is expected to deepen our understanding of metastatic biology and support the development of more effective precision-based anticancer strategies.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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# Chapter 9

## Cytoskeletal and Cell Junction Disorders in Infertility

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**Abstract**

Infertility may arise not only from intrinsic germ-cell abnormalities but also from defects in the somatic microenvironment that supports gametogenesis. In the testis, actin filaments, microtubules, intermediate filaments, and specialized junctional complexes maintain blood-testis barrier integrity, Sertoli-germ cell adhesion, and germ-cell transport. In the ovary, actin-rich transzonal projections, gap junctions, and the oocyte cytoskeleton coordinate cumulus-oocyte communication and meiotic maturation. This chapter reviews normal embryological and histological organization, relevant cytoskeletal and junctional systems, and their regulatory pathways, then examines disruption by oxidative stress, inflammation, aging, endocrine-metabolic disorders, and genetic defects. Diagnostic approaches, experimental models, and current therapeutic strategies are critically evaluated, emphasizing cytoskeletal and cell-junction dysfunction as a shared but tissue-specific axis of infertility.

**Keywords:** infertility; cytoskeleton; cell junctions; blood-testis barrier; cumulus-oocyte complex; transzonal projections.

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## 1. Introduction

Human fertility depends on lifelong structural and functional interactions between germ cells and the surrounding somatic cells. Cytoskeletal networks of actin filaments, microtubules, and intermediate filaments, along with cell junctions such as tight junctions, adherens junctions, gap junctions, and desmosomes, underpin these interactions. Maintenance of cell polarity, intracellular transport, intercellular signaling, germ-cell development, and tissue integrity depends on the coordinated function of these structures. Accordingly, infertility should be considered not only in relation to hormonal imbalances, anatomical abnormalities, or reduced gamete numbers, but also as a condition in which disruption of cellular architecture and intercellular communication within reproductive tissues may contribute to impaired reproductive function (Luaces et al., 2023; Marchais et al., 2022; Stanton, 2016; Xie et al., 2023).

Infertility represents a major and increasing global reproductive health burden (Feng et al., 2025; Liu et al., 2025). Although male and female infertility present with distinct clinical phenotypes, maintenance of gametogenesis in both systems requires tightly regulated communication between germ cells and supporting somatic cells. In the testis, the blood-testis barrier (BTB) formed by Sertoli cells provides an immunologically and biochemically regulated microenvironment for developing germ cells. In the ovary, the cumulus-oocyte complex (COC), transzonal projections (TZPs), and gap junctions support the metabolic and molecular exchange required for oocyte growth, meiotic arrest, and acquisition of developmental competence (Luaces et al., 2023; Marchais et al., 2022; Xie et al., 2023).

Dysregulated cytoskeletal remodeling or loss of cell-junction integrity can alter the histological organization and cellular functions of reproductive tissues. Oxidative stress, chronic inflammation, aging, metabolic disease, and certain genetic defects can disrupt actin and microtubule dynamics as well as the expression and subcellular localization of junctional proteins. In males, these changes are associated with increased BTB permeability, weakened Sertoli-germ cell adhesion, and disordered spermatogenesis; in females, they are associated with impaired cumulus-oocyte communication, meiotic spindle defects, and reduced oocyte developmental competence. Thus, although different etiologies produce tissue-specific outcomes, disruption of cytoskeletal organization and intercellular communication constitutes a shared pathobiological axis (Stanton, 2016; Xie et al., 2023).

This chapter summarizes the normal embryological and histological organization of the male and female reproductive systems and examines the cytoskeletal networks, cell junctions, and major regulatory signaling mechanisms involved in maintaining fertility. Structural alterations arising in the context of oxidative stress and inflammation, aging, endocrine-metabolic disorders, and genetic defects are then

evaluated. The paper also discusses the extent to which current therapies can target these abnormalities, the clinical limitations of experimental approaches, and future directions for translational research.

## 2. Normal Histology & Embryological Development

### 2.1 Male Reproductive System: Embryological Development and Histological Organization

Development of the male reproductive system begins with differentiation of the bipotential gonad toward the testicular pathway. Primordial germ cells migrate to the gonadal ridge and interact with developing somatic gonadal cells. In the XY gonad, early supporting cells initiate sex-determining region Y (SRY) expression, followed by activation of SRY-box transcription factor 9 (SOX9), which directs their differentiation into the Sertoli cell lineage and promotes testis cord formation. During fetal development, Sertoli cells support the organization of germ cells within the cords and establishment of the testis-specific microenvironment; postnatally, they also contribute to organization of the seminiferous epithelium and formation of the BTB. Single-cell studies of human gonadal development have shown that differentiation of germ-cell and somatic-cell lineages is temporally and molecularly coordinated (Garcia-Alonso et al., 2022; Gao et al., 2024).

The seminiferous tubule is the principal functional unit of the adult testis. Sertoli cells rest on the basement membrane and extend cytoplasmic processes from the basal region toward the lumen, surrounding germ cells at different stages of development. Spermatogonia reside in the basal compartment, whereas meiotic and postmeiotic germ cells progress toward the adluminal compartment. Sertoli cells coordinate nutritional support, paracrine regulation, adhesion, germ-cell transport, and spermiation. Leydig cells located in the interstitial space between seminiferous tubules produce testosterone, providing endocrine support for spermatogenesis (Li et al., 2024).

One of the defining features of normal testicular histology is the BTB formed between adjacent Sertoli cells. The BTB divides the seminiferous epithelium into basal and adluminal compartments, thereby creating an immunologically privileged and compositionally regulated environment for meiotic and postmeiotic germ cells. The barrier, however, is not static. During the transit of preleptotene spermatocytes into the adluminal compartment, disassembly of pre-existing junctions and assembly of new junctions occur in a coordinated manner. This dynamic remodeling requires precise coordination between junctional complexes and the actin and microtubule networks (Mruk & Cheng, 2015; Stanton, 2016; Wen et al., 2018).

### 2.2 Female Reproductive System: Embryological Development and Histological Organization

Development of the female gonad begins with differentiation of the bipotential gonad toward the ovarian pathway. In the XX gonad, Wnt family member 4 (WNT4)- and R-spondin 1 (RSPO1)-mediated activation of β-catenin signaling promotes ovarian somatic-cell differentiation and suppresses the testis-specific developmental program. After migrating to the gonadal ridge, primordial germ cells proliferate mitotically to form oogonia; entry of oogonia into meiosis I gives rise to primary oocytes. Primary oocytes arrest at the diplotene stage of prophase I during fetal life and become surrounded by pregranulosa cells to form primordial follicles. This early association between germ cells and somatic cells provides the structural basis for subsequent follicular development. Single-cell and spatial transcriptomic studies of human gonadal development support the presence of spatially organized pregranulosa-cell populations within the developing ovary and their potential paracrine interactions with oocytes (Garcia-Alonso et al., 2022).

In the adult ovary, folliculogenesis is a structural and functional maturation process that progresses from the primordial follicle through the primary, secondary, antral, and preovulatory stages. The oocyte develops within an organized microenvironment composed of granulosa cells and, more peripherally, theca cells. In the antral follicle, granulosa cells undergo functional specialization into mural granulosa cells lining the follicular wall and cumulus cells surrounding the oocyte. Cumulus cells provide substrates required for oocyte energy metabolism, contribute to regulation of meiotic status, and support acquisition of oocyte developmental competence (Richani et al., 2021; Turathum et al., 2021).

The cumulus-oocyte complex (COC) is the central functional unit of this organization. Transzonal projections (TZPs) extending from cumulus cells through the zona pellucida toward the oocyte, together with gap junctions, support direct cellular and molecular communication between the oocyte and surrounding somatic cells. This communication contributes to oocyte growth, meiotic control, and acquisition of developmental competence (Marchais et al., 2022; Xie et al., 2023).

Despite their anatomical and functional differences, germ-cell development in both reproductive systems depends on the structural integrity and communication capacity of the tissue-specific microenvironment established by supporting somatic cells.

## 3. Molecular, Histological and Cellular Structure and Related Major Signaling Pathways

### 3.1 Cytoskeletal and Cell Junction Organization in the Male Reproductive System

Successful spermatogenesis requires coordinated Sertoli–germ cell interactions and dynamic cytoskeletal remodeling that preserve epithelial polarity while supporting germ-cell transport and spermiation (Stanton, 2016; Wen et al., 2018).

The BTB is not composed solely of tight junctions. Tight junctions containing claudin-11, occludin, and zonula occludens-1 (ZO-1) are integrated within the same region as actin-rich basal ectoplasmic specializations, gap junctions, and desmosomes between Sertoli cells. N-cadherin-β-catenin and nectin-2-afadin complexes link to the actin cytoskeleton and contribute to the mechanical stability of basal ectoplasmic specializations, whereas connexin 43 (Cx43)-containing gap junctions support direct intercellular communication between Sertoli cells. This distinctive organization is one of the key structural features that differentiates the BTB from other blood-tissue barriers (Luaces et al., 2023; Mruk & Cheng, 2015).

Actin filaments form the principal cytoskeletal scaffold of basal and apical ectoplasmic specializations, supporting germ-cell adhesion and spermatid orientation. Microtubules form polarized tracks along the Sertoli cell and contribute to the transport of germ cells and organelles. Vimentin intermediate filaments support the mechanical integrity and perinuclear organization of Sertoli cells. These networks do not function independently; endocytosis, recycling, and redistribution of junctional proteins depend on coordinated actin-microtubule interactions. Therefore, normal expression of junctional proteins alone does not ensure barrier function if their appropriate subcellular localization is not maintained (Luaces et al., 2023; Wen et al., 2018).

Sertoli-cell functions are regulated by endocrine and local signals. Follicle-stimulating hormone (FSH) signaling through the FSH receptor (FSHR) activates the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway and supports the metabolic and trophic functions of Sertoli cells, while extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) and phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathways may also contribute to FSH responses depending on developmental stage and cellular context. Testosterone signaling through the androgen receptor in Sertoli cells is required for maturation of the BTB and junctional complexes, completion of meiosis, spermiogenesis, and spermiation. In addition, the transforming growth factor beta 3 (TGF-β3)-p38 MAPK axis can regulate BTB dynamics by altering the abundance and subcellular distribution of junctional proteins such as occludin. The effects of these signaling mechanisms depend on developmental stage, the phase of the

seminiferous epithelial cycle, and the duration of stimulation. Pathways that mediate physiological barrier remodeling may disrupt junctional integrity when aberrantly or excessively activated (Li et al., 2024; Mruk & Cheng, 2015).

Cytoskeletal remodeling also extends to spermiogenesis. The acrosome-acroplaxome-manchette complex coordinates sperm-head shaping: the acroplaxome provides an actin-associated interface linking the developing acrosome to the nuclear envelope, whereas the transient microtubule-rich manchette contributes to nuclear remodeling and cargo transport. Flagellar formation depends on the microtubule-based axoneme and associated peri-axonemal structures. Consequently, defects in these cytoskeletal systems can produce abnormal head morphology, impaired flagellar architecture, and reduced motility (He et al., 2025; Zhou et al., 2024).

### 3.2 Cytoskeletal and Cell Junction Organization in the Female Reproductive System

Oocyte development depends on bidirectional communication with granulosa and cumulus cells, which provide metabolic support while responding to oocyte-derived regulatory signals (Richani et al., 2021; Turathum et al., 2021).

TZPs are predominantly actin-rich cytoplasmic extensions that traverse the zona pellucida and reach the oolemma. In mammalian follicles, particularly in experimental models, connexin 37 (Cx37)-containing gap junctions at the oocyte-somatic cell interface mediate communication between the oocyte and surrounding cumulus cells, whereas Cx43 is prominent at junctions among granulosa and cumulus cells. This differential distribution supports complementary but functionally non-equivalent roles of these connexins within the follicular communication network (Marchais et al., 2022; Xie et al., 2023).

The oocyte cytoskeleton is dynamically reorganized during meiotic maturation. Microtubules assemble the meiotic spindle and support chromosome alignment and segregation, whereas actin networks contribute to spindle positioning, cortical organization, asymmetric cytokinesis, and polar-body extrusion. Because mammalian oocytes lack conventional centrosomes, spindle assembly depends on the self-organization and maturation of microtubule-organizing centers and associated regulatory proteins. Coordination between actin and microtubule networks is therefore essential for accurate chromosome segregation; disruption can result in spindle abnormalities, chromosome misalignment, maturation arrest, or aneuploidy (Dunkley & Mogessie, 2023; Gu et al., 2025; Wu et al., 2024).

The natriuretic peptide precursor C (NPPC)/natriuretic peptide receptor 2 (NPR2)-cyclic guanosine monophosphate (cGMP) axis plays a central role in maintaining meiotic arrest. cGMP generated in mural granulosa and cumulus cells reaches the oocyte through gap junctions and inhibits phosphodiesterase 3A

(PDE3A) within the oocyte. This limits degradation of oocyte-derived cAMP, thereby maintaining high intracellular cAMP levels and sustaining prophase I arrest. Following the luteinizing hormone (LH) surge, follicular cGMP levels decrease, inhibition of PDE3A is relieved, oocyte cAMP declines, and meiosis resumes. Thus, transfer of cGMP from somatic cells to the oocyte and regulation of intra-oocyte cAMP homeostasis are distinct but sequential events (Jaffe & Egbert, 2017).

Oocyte-derived growth differentiation factor 9 (GDF9) and bone morphogenetic protein 15 (BMP15) regulate cumulus-cell differentiation, metabolic activity, and expansion through SMAD-mediated signaling pathways. The preovulatory LH surge stimulates mural granulosa cells to produce epidermal growth factor (EGF)-like ligands. Activation of the epidermal growth factor receptor (EGFR)-MAPK pathway in cumulus cells by these ligands contributes to cumulus expansion and to a controlled reduction in gap-junctional communication. As a result, somatic-cell-derived signals that maintain meiotic arrest decline, allowing oocyte maturation to progress (Jaffe & Egbert, 2017; Turathum et al., 2021; Xie et al., 2023).

## 4. Disease Mechanisms & Histopathological/Structural Changes

### 4.1 Oxidative Stress and Inflammation

Oxidative stress and chronic inflammation can disrupt cellular homeostasis in male and female reproductive tissues, thereby compromising cytoskeletal organization and cell-junction integrity. Excess reactive oxygen species can damage membrane lipids, proteins, and nucleic acids, while the inflammatory microenvironment can impair mechanisms that regulate cell polarity, adhesion, and intercellular communication. Nevertheless, in many infertility settings, oxidative stress is better regarded not as an isolated primary cause but as a common injury mechanism that interacts with hormonal, vascular, metabolic, and inflammatory disturbances (Luaces et al., 2023; von Mengden et al., 2020).

In the male reproductive system, the Sertoli-cell actin network and the junctional complexes that form the BTB are major targets of oxidative and inflammatory injury. Alterations in the expression or subcellular localization of BTB junctional proteins, together with disruption of Filamentous actin (F-actin) organization, can weaken barrier integrity and increase permeability (Luaces et al., 2023). In varicocele, the combined effects of hyperthermia, hypoxia, oxidative stress, and inflammation can impair Sertoli-cell function and the testicular microenvironment, leading to germ-cell apoptosis, disorganization of the seminiferous epithelium, and reduced sperm quality (Ma et al., 2026). However, much of the evidence regarding these molecular mechanisms derives from experimental and preclinical studies.

In the female reproductive system, oxidative stress can affect both junctional communication between the cumulus cells and the oocyte and the meiotic

cytoskeleton of the oocyte. In experimental bovine COC models, attenuation of oxidative stress has been shown to preserve the number of actin-rich TZPs and cumulus-oocyte gap-junctional communication, accompanied by increased intra-oocyte glutathione levels and improved developmental competence (Khatun et al., 2024). In mouse oocytes, hydroxyl radical-mediated oxidative injury disrupted the organization of spindle microtubules and chromosome alignment at the metaphase plate (Shaeib et al., 2013). Although associations between redox and metabolic markers in human cumulus cells and oocyte or embryo development have been reported, these markers have not yet been sufficiently standardized or validated for routine clinical use (von Mengden et al., 2020).

### 4.2 Aging

Aging is accompanied by progressive accumulation of cellular damage and declining maintenance and repair capacity in reproductive tissues. Mitochondrial dysfunction, oxidative stress, low-grade inflammation, and cellular senescence affect not only gametes but also the somatic cells that support them. Consequently, organization of the seminiferous epithelium and function of the interstitial microenvironment may deteriorate in the testis, while the follicular microenvironment and the structural and functional integrity of the oocyte may decline in the ovary. However, the timing and reproductive consequences of aging differ between the sexes: oocyte reserve and quality are affected earlier and more prominently in females, whereas changes in males generally occur more gradually (Dong et al., 2022; Wang et al., 2023).

In the aging testis, seminiferous tubule disorganization, germ-cell loss, and reduced efficiency of spermatogenesis may occur (Dong et al., 2022). These changes are accompanied by alterations in Sertoli-cell cytoskeletal organization. In aged mice, Sertoli cells have been shown to lose polarity and become flattened, with F-actin distribution extending from cell-cell junctions and the periphery of spermatid heads toward the luminal cytoplasm, together with loss of normal vimentin organization within cellular processes. In severely thinned seminiferous epithelium, re-emergence of cytokeratin expression, which is not prominent in normal mature Sertoli cells, has also been interpreted as a marker of age-related cellular remodeling (Tanemura et al., 1994). In aged human testes, increased vimentin immunoreactivity and re-expression of keratin have been reported in Sertoli cells within germ-cell-depleted and severely damaged tubules (de Miguel et al., 1997). These findings indicate that aging can alter the actin and intermediate-filament networks that support Sertoli-cell mechanical integrity, polarity, and seminiferous epithelial organization.

In the female reproductive system, the most prominent consequences of aging are depletion of the oocyte reserve and deterioration of oocyte quality (Wang et al., 2023). Age-related cohesin loss predisposes to premature separation of sister chromatids, whereas the spindle-associated F-actin network helps preserve chromatid cohesion by constraining microtubule-generated separating forces. This F-actin network has been shown to become disrupted in oocytes from aged mice (Dunkley & Mogessie, 2023). In oocytes obtained from women of advanced reproductive age, disorganized meiotic spindle microtubules and chromosome displacement from the metaphase plate have been observed more frequently (Battaglia et al., 1996). In middle-aged mice, researchers have also reported reductions in the number of actin-rich transzonal projections, connexin gene expression, and molecular transfer between cumulus cells and the oocyte (Ishikawa-Yamauchi et al., 2024).

Because much of the mechanistic evidence, particularly for oocyte F-actin and cumulus–oocyte communication, derives from murine models, direct validation in human reproductive tissues remains necessary.

### 4.3 Endocrine and Metabolic Disorders

Endocrine and metabolic disorders can affect not only hormonal signaling within reproductive tissues but also the cellular microenvironment that supports germ-cell development. Metabolic and hormonal alterations associated with conditions such as diabetes mellitus, hypogonadism, and polycystic ovary syndrome (PCOS) can adversely affect somatic-cell function, intercellular communication, and gamete development. Therefore, fertility impairment in these disorders cannot be explained solely by changes in circulating hormone levels (Li et al., 2024; Sayutti et al., 2022; Zhang et al., 2025).

In the male reproductive system, chronic hyperglycemia can impair Sertoli-cell function and BTB integrity through oxidative stress, inflammation, and accumulation of advanced glycation end products. Under diabetic conditions, reduced expression of junctional proteins such as Cx43 and ZO-1 in Sertoli cells and increased BTB permeability have been reported. These changes may be accompanied by impaired Sertoli-cell metabolism, germ-cell apoptosis, and defective spermatogenesis (Zhang et al., 2025). In hypogonadal states, insufficient FSH and testosterone signaling may adversely affect the spermatogenesis-supporting functions of Sertoli cells and normal organization of the BTB (Li et al., 2024).

In PCOS, hyperandrogenism, insulin resistance, and chronic inflammation can alter the metabolic and molecular characteristics of the follicular microenvironment. In luteinized granulosa cells obtained from women with PCOS, reduced Cx43 expression and impaired gap-junctional intercellular communication have been

demonstrated; increased miR-130b expression has been proposed to contribute to this change through post-transcriptional suppression of Cx43 (Jiang et al., 2020). Abnormalities in mitochondrial ultrastructure, Adenosine triphosphate (ATP) production, membrane potential, and oxidative status have also been described in granulosa cells from women with PCOS (Xie et al., 2024). Changes in cumulus-cell gene-expression profiles have likewise been associated with oocyte quality, although the clinical validity of these molecular markers has not yet been adequately established for routine use (Sayutti et al., 2022).

### 4.4 Genetic Disorders

Pathogenic variants affecting proteins required for cytoskeletal organization can impair sperm flagellar architecture or oocyte spindle formation. However, gene–disease relationships should be supported by both patient-level and functional evidence.

A prominent example of cytoskeleton-related genetic defects in male infertility is the multiple morphological abnormalities of the sperm flagella (MMAF) phenotype. Pathogenic variants in genes involved in the organization of axonemal or peri-axonemal structures, including dynein axonemal heavy chain 1 (DNAH1)**,** cilia and flagella associated protein 43 (CFAP43), and cilia and flagella associated protein 44 (CFAP44), can result in short, coiled, irregular, or absent flagella and severe asthenoteratozoospermia. These genetic defects disrupt the microtubule-based structural organization of the sperm flagellum, leading to a marked reduction in motility and male infertility (Zhou et al., 2024).

In female infertility, pathogenic tubulin beta 8 class VIII (TUBB8) variants have been associated with maturation arrest, fertilization failure, or early embryonic developmental defects by disrupting microtubule organization and meiotic spindle formation in human oocytes (Cao et al., 2021). Variants in HAUS augmin like complex subunit 6 (HAUS6)**,** kinesin family member 11 (KIF11), and kinesin family member 18A (KIF18A), which contribute to spindle bipolarization in human oocytes, have also been identified in selected infertile patients with oocyte or embryonic developmental defects and have been shown functionally to disrupt spindle organization (Wu et al., 2024). The Cytoskeleton-associated protein 5 (CKAP5)-encoded protein is another regulator required for maturation of microtubule-organizing centers and assembly of the meiotic spindle in human and mouse oocytes (Gu et al., 2025). In addition, human genetic data and experimental models link *CKAP5* deficiency to diminished ovarian reserve and premature ovarian insufficiency; however, more clinical evidence is needed before *CKAP5* variants can be considered an established monogenic cause of oocyte maturation failure comparable to *TUBB8* (Hu et al., 2025).

## 5. Clinical Manifestations & Diagnostic Biomarkers

### 5.1 Male Infertility

The clinical manifestations of cytoskeletal and cell-junction disorders in male infertility vary according to the affected cell type and structure. Processes that compromise the integrity of the seminiferous epithelium and the BTB may disrupt different stages of spermatogenesis and contribute to reduced sperm counts or azoospermia. In contrast, defects affecting the structural organization of the sperm head, acrosome, midpiece, or flagellum are more commonly associated with teratozoospermia and asthenozoospermia. In particular, multiple morphological abnormalities of the sperm flagella (MMAF) represent a severe asthenoteratozoospermia phenotype. Semen analysis can identify abnormalities in sperm concentration, motility, and morphology but cannot, by itself, define the underlying cytoskeletal or molecular defect (Arora et al., 2024; Zhou et al., 2024).

Histopathological examination of testicular tissue may reveal various patterns of spermatogenic impairment, including hypospermatogenesis, maturation arrest, Sertoli-cell-only pattern, and tubular atrophy; however, these findings are not specific to cytoskeletal or cell-junction disorders. In clinically obtained testicular tissue, the expression and subcellular localization of claudin-11, occludin, ZO-1, Cx43, and associated cytoskeletal components can be investigated by immunohistochemistry or immunofluorescence. Although these markers are valuable for research-based assessment of the structural and functional integrity of the BTB, they are not routinely used as clinical diagnostic biomarkers (Luaces et al., 2023).

In patients with marked sperm morphological abnormalities, genetic analysis may help identify the molecular defect underlying the structural phenotype. Numerous axonemal and peri-axonemal genes, including *DNAH1*, *CFAP43*, and *CFAP44*, have been associated with MMAF; Dpy-19 like 2 (DPY19L2) with globozoospermia; and Aurora kinase C (AURKC) with macrozoospermia. Identification of pathogenic variants in these genes can help define the molecular basis of the morphological phenotype and inform genetic counseling. Nevertheless, interpret genetic findings in the context of the semen phenotype, family history, inheritance pattern, and functional evidence supporting variant pathogenicity (Arora et al., 2024; Zhou et al., 2024).

### 5.2 Female Infertility

In women, cytoskeletal and meiotic organizational defects may present as recurrent germinal-vesicle or metaphase I arrest, abnormal metaphase II oocytes, fertilization failure, or early embryonic developmental arrest. Although oocyte maturation stage and morphology can be assessed in assisted reproduction

laboratories, morphology alone does not reveal meiotic spindle organization or the underlying molecular defect. Pathogenic variants in genes such as *TUBB8*, PAT1 homolog 2 (PATL2), and thyroid hormone receptor interactor 13 (TRIP13) have been associated with recurrent oocyte maturation defects, while other genetic abnormalities may cause failure at the stages of fertilization or early embryonic development. Therefore, in recurrent and pronounced defects of oocyte maturation, fertilization, or early embryo development, genetic analysis can contribute to identifying the molecular basis of the phenotype (Cao et al., 2021; Sang et al., 2021).

Because cumulus cells are in close metabolic and molecular communication with the oocyte, they represent a potential source of biomarkers that may indirectly reflect oocyte developmental competence. In assisted reproduction, associations between cumulus-cell gene-expression profiles and oocyte maturation, embryo quality, and pregnancy outcomes have been investigated, and candidate genes including hyaluronan synthase 2 (HAS2), prostaglandin-endoperoxide synthase 2 (PTGS2), Gremlin 1, DAN family BMP antagonist (GREM1), Versican (VCAN), GDF9, and BMP15 have been identified. However, these associations are not consistent across studies and may be influenced by patient characteristics, ovarian stimulation protocols, and analytical methods. Thus, although cumulus-cell-derived molecular markers are promising, they are not currently validated biomarkers for routine oocyte or embryo selection (Massoud et al., 2024; Sayutti et al., 2022). Next-generation sequencing and high-resolution cellular imaging approaches may contribute to more detailed characterization of these structural and molecular phenotypes in selected cases; however, they are not yet part of routine clinical diagnosis.

## 6. Experimental Models

### 6.1 *In Vitro* Models

Sertoli-cell culture systems are among the principal in vitro models used to examine the cellular and molecular properties of the BTB under controlled conditions. In systems in which primary Sertoli cells are cultured on permeable supports, barrier integrity, barrier permeability, and organization of junctional proteins can be evaluated. These models allow investigation of how BTB components such as claudin-11, occludin, ZO-1, and the actin network respond to hormonal, inflammatory, or toxic stimuli. However, when germ cells, Leydig cells, peritubular myoid cells, and interstitial components are not included, Sertoli-cell cultures cannot fully reproduce the complex testicular microenvironment (Mruk & Cheng, 2015; Wu et al., 2021).

In the female reproductive system, COC culture and in vitro oocyte maturation models are widely used to investigate structural and functional communication between the oocyte and cumulus cells. These systems enable controlled assessment

of processes including organization of actin-rich TZPs, gap-junction-mediated molecular transfer, cGMP-cAMP-mediated meiotic control, cumulus expansion, and meiotic progression of the oocyte. However, removal of the COC from the follicular environment can alter the physiological context of oocyte-somatic-cell communication; moreover, developmental and metabolic differences between rodent and human follicles limit direct translation of experimental findings to human fertility (Jaffe & Egbert, 2017; Santiquet et al., 2017; Xie et al., 2023).

### 6.2 Animal Models

Rodent models enable investigation of cytoskeletal organization and cell junctions in reproductive tissues within an in vivo tissue context. In males, experimental models of conditions such as diabetes and varicocele are used to evaluate histological and molecular changes in Sertoli-cell junctions, BTB integrity, and seminiferous epithelial organization (Ma et al., 2026; Zhang et al., 2025). In females, rodent models with PCOS-like features induced by androgens, anti-Müllerian hormone, or letrozole allow investigation of impaired follicular development and oocyte-somatic-cell interactions (Stener-Victorin, 2022). Nevertheless, differences in reproductive physiology between species, and the fact that experimental models reproduce only selected features of human disease, limit the direct translation of these findings to clinical infertility (Stener-Victorin, 2022).

### 6.3 Organoid and Three-Dimensional Culture Systems

Testicular organoids and three-dimensional culture systems aim to reproduce interactions among Sertoli, germ, Leydig, and peritubular cells, as well as the three-dimensional organization of the testicular microenvironment, more closely than conventional two-dimensional cultures. These models may allow controlled investigation of testicular morphogenesis, the spermatogonial stem-cell niche, organization of cell junctions, and germ-cell development. However, reliable and reproducible reconstruction of seminiferous tubule architecture and fully functional human spermatogenesis remains a major limitation, and substantial variability exists among cell sources, matrices, and culture protocols (Alves-Lopes & Stukenborg, 2018).

In three-dimensional ovarian culture systems, researchers culture isolated follicles within collagen, alginate, or similar matrices to preserve follicular three-dimensional architecture, granulosa-oocyte interactions, and cell-extracellular matrix relationships. These systems allow controlled assessment of follicular growth, oocyte maturation, and follicular-cell function. However, the culture matrix can influence follicular growth and structural integrity, and current three-dimensional systems do not fully reproduce all components of the native ovarian

microenvironment (Zuccotti et al., 2015). Accordingly, model selection should be guided by the research question, and clinically relevant findings should ultimately be validated in human samples.

## 7. Current Therapeutic Approaches

Current infertility treatments generally address the underlying disorder or circumvent gamete dysfunction through assisted reproductive technologies rather than directly targeting cytoskeletal or junctional defects.

In male infertility associated with conditions such as diabetes and varicocele, correction of the metabolic, oxidative, and inflammatory microenvironment may help reduce secondary damage within testicular tissue. Experimental studies have reported protective effects of interventions targeting oxidative stress and inflammation on Sertoli-cell function, BTB integrity, and spermatogenesis. However, much of this evidence remains preclinical, and no established clinical therapy directly targets cytoskeletal or junctional proteins (Ma et al., 2026; Zhang et al., 2025).

In female infertility, one of the approaches most closely related to cytoskeletal and intercellular communication mechanisms is the refinement of in vitro maturation (IVM) protocols. In pre-IVM approaches, controlled modulation of cAMP and cGMP levels aims to delay spontaneous meiotic resumption, supporting communication between the oocyte and cumulus cells and giving the oocyte additional time to acquire developmental competence. Biphasic IVM strategies based on these principles are being evaluated in experimental and translational studies, although their indications and protocols continue to evolve (Jaffe & Egbert, 2017; Richani et al., 2021).

Assisted reproductive technologies do not correct the underlying structural defect but may help overcome the functional consequences of certain cytoskeleton-related gamete abnormalities. For example, fertilization can be achieved by intracytoplasmic sperm injection (ICSI) in some cases of MMAF characterized by severe sperm motility and flagellar abnormalities, although outcomes may vary according to the underlying genetic defect. In pathogenic variants of genes such as *TUBB8* that disrupt meiotic spindle organization in the oocyte, phenotypes may include maturation arrest, fertilization failure, or early embryonic developmental abnormalities, and assisted reproductive outcomes may vary accordingly (Cao et al., 2021; Zhou et al., 2024).

## 8. Future Perspectives

Although understanding of the role of cytoskeletal and cell-junction disorders in infertility is increasing, much of the available evidence comes from animal models

and in vitro studies. Future work should validate experimentally defined mechanisms in human testicular tissue, ovarian follicles, and COCs and relate them to clinical infertility phenotypes. Such assessments should integrate protein expression with subcellular localization, cytoskeletal organization, and functional outcomes.

Single-cell RNA sequencing, spatial transcriptomics, proteomics, and advanced imaging methods provide new opportunities to define cell-type-specific alterations within reproductive tissues. Cellular atlases generated during human gonadal development demonstrate the potential of these technologies; applying similar approaches to infertility may help distinguish primary disease mechanisms from secondary changes (Garcia-Alonso et al., 2022).

Future biomarkers may include cumulus-cell transcripts and other molecular indicators related to the cytoskeleton or cell junctions. However, before they can be incorporated into clinical practice, they must be validated in independent cohorts and shown to correlate with meaningful reproductive outcomes, including fertilization, embryo development, pregnancy, and live birth (Massoud et al., 2024). A key priority is to determine whether cytoskeletal and junctional abnormalities are primary drivers of infertility or secondary consequences of disease, as this distinction will be critical for identifying reliable biomarkers and tissue-specific therapeutic targets.

## 9. Conclusions

Infertility is associated not only with hormonal and anatomical abnormalities but also with disruption of the structural and functional communication between germ cells and their supporting somatic cells. Cytoskeletal networks and cell junctions play fundamental roles in maintaining reproductive tissue architecture, preserving cell polarity, and regulating the intercellular communication required for gamete development.

In the testis, the BTB and ectoplasmic specializations support organization of the seminiferous epithelium and germ-cell development, whereas in the ovary, the COC, TZPs, and a gap-junction network containing Cx37 and Cx43 maintain metabolic and molecular communication between the oocyte and somatic cells. Although these structures are anatomically and functionally distinct, both systems depend on the dynamic and coordinated organization of cell junctions and cytoskeletal networks.

Oxidative stress, inflammation, aging, endocrine-metabolic disorders, and genetic defects can disrupt this organization through different mechanisms. The resulting consequences may include altered cell polarity, loss of junctional integrity, impaired barrier function, reduced gap-junctional communication, and disruption of germ-cell-somatic-cell coordination. However, the nature of these changes and their contribution to infertility vary by disease context, cell type, and sex. In conclusion,

cytoskeletal organization and cell junctions provide an important common framework for understanding the cellular pathogenesis of male and female infertility. Evaluation of these structures in a tissue- and cell-type-specific manner is essential for a more integrated and accurate understanding of the mechanisms underlying infertility.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Declaration of AI:** ChatGPT (OpenAI) was used solely for English language editing and improving readability.

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