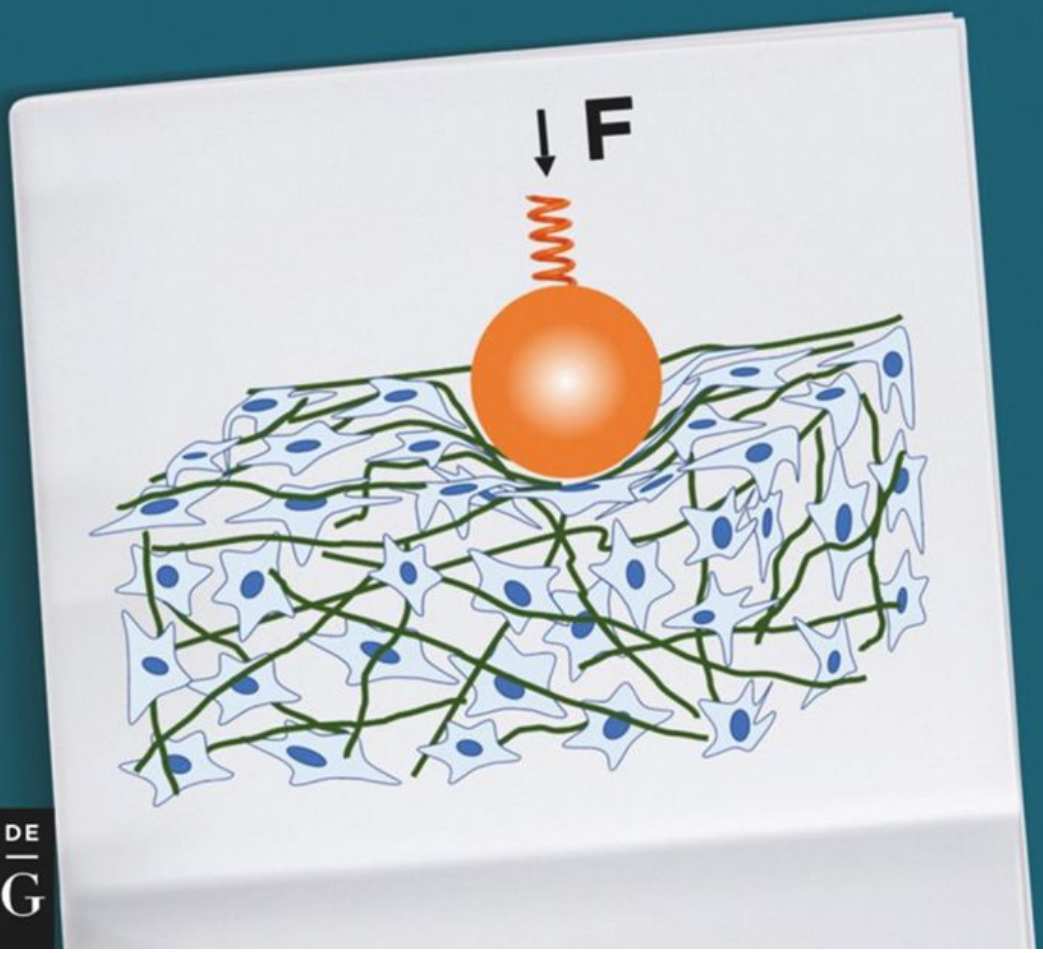
DE GRUYTER STEM

MECHANICS OF CELLS AND TISSUES IN DISEASES

BIOMEDICAL METHODS

Edited by Malgorzata Lekka, Daniel Navajas, Alessandro Podestà and Manfred Radmacher



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Wolfgang H. Goldmann

4.2 The Cytoskeleton

Adherent cells are anchored via focal adhesions to the extracellular matrix, which is essential for force transduction, cell spreading, and migration. Focal adhesions consist of clusters of transmembrane adhesion proteins of the integrin family and numerous intracellular proteins, including talin and vinculin. They link integrins to actin filaments and are key players of focal adhesions that build up a strong physical connection for transmitting forces between the cytoskeleton and the extracellular matrix. These proteins consist of a globular head and a tail domain that undergo conformational changes from a closed, autoinhibited conformation in the cytoplasm to an open, active conformation in focal adhesions, which is regulated by phosphorylation.

4.2.1 Actin Cytoskeleton

Over the years, much research has provided information on the cellular function of the cytoskeleton, which has helped in understanding the many aspects of cell behavior. Components of the cytoskeletal network are major regulators of processes as diverse as establishing and maintaining gross cell morphology, polarity, transduction of force, motility, and adhesion to matrix components and cells. The cytoskeleton has long been proposed to be involved in the organization/reorganization of reporters in the plasma membrane. It is, therefore, critical to cell recognition mechanisms for many types of associations. These can range from tissue formation to the immune killing of foreign cells. Hence, the association of cytoskeletal elements with membrane components became a paradigm for signal transduction to the cytoplasm from the cell surface and vice versa. Interaction sites for membrane proteins with the interior of the cell are also key integration sites for transmitting signals to several pathways, eliciting pleiotypic responses of cells to signals. Thus, membrane–cytoskeletal complexes are mediators of crosstalk between receptors. Cell surface receptors for diverse ligands, including growth factors and hormones, and cell–matrix and cell–cell adhesion proteins, are transmembrane linked to microfilaments, which in turn interact with both microtubules and intermediate filaments (IFs). These interactive systems of membranes, with all of the cytoskeletal arrays, can elicit the global responses of cells to ligands such as mitogens, which evoke major morphological perturbations (Carraway and Carraway, 2000).

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The cytoskeleton is a highly dynamic, multifunctional network that connects all compartments of the cell in a three-dimensional space. This intracellular network provides eukaryotic cells with structural support to maintain cell shape and directional locomotion. At the same time, it provides the opportunity for active, directed transport, such as organelles or the separation of chromosomes in mitosis. In addition to actin fibers, the cytoskeleton consists of two other types of protein filaments, microtubules, and intermediate filaments (IFs). All three comprise dynamic protein components that polymerize into spiral-shaped fiber bundles (Figure 4.2.1).

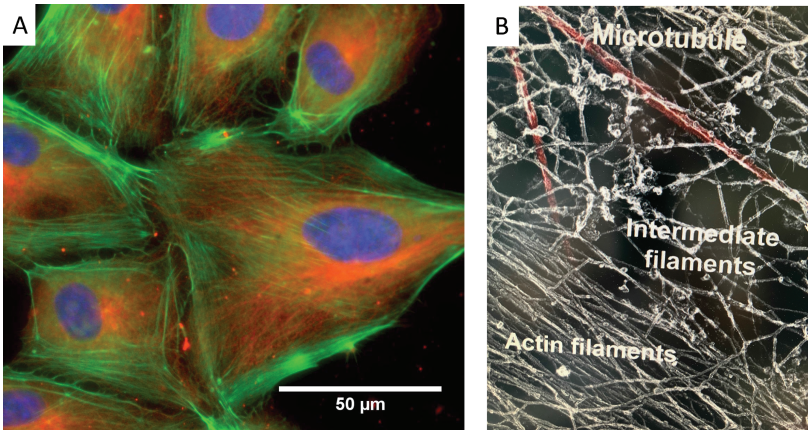

Figure 4.2.1: Filament types of the cell cytoskeleton. (A) HUVEC (human umbilical vein endothelial cells) taken by an 60x oil immersion objective. Green, F-actin (LifeAct-TagGFP2 protein); Red, Tubulin (Monoclonal anti-alpha-Tubulin); Blue, Nuclei (DAPI staining using ibidi mounting medium) adapted and taken from www.ibidi.com with permission. (B) Electron micrograph of the three filament types from a permeabilized cell. After freezing and sublimation of water, the structures were coated with platinum. Microtubules were highlighted in red (adapted and taken from Pollard and Cooper (2009) with permission).

Actin filaments (F-actin), with their flexible, double-helical structure of polymerized globular monomers (G-actin) have a diameter of 7–9 nm. They are found below the plasma membrane as a network (cortical actin) and also in the cytoplasm as discrete fiber bundles (stress fibers) starting from adhesion complexes to the membrane. This type of filament also shows orientation, as polymerization takes place at both ends, but at different rates. The slower growing *pointed end* (minus end) points toward the interior of the cell and the *barbed end* (plus end) polymerizes faster toward the plasma membrane. The actin monomers follow the so-called *treadmill* mechanism (Pollard and Mooseker, 1981), as ATP-bound G-actin attaches preferentially to the plus end through weak non-covalent bonds, while monomers bound to dephosphorylated ADP detach at the minus end of the filament (Carlier and Pantaloni, 1997). At constant G-actin concentrations in the cell, dynamic restructuring of filaments takes place by this

mechanism, while the length remains constant. However, some toxins found in sponges and fungi affect the dynamics of actin fibers and are therefore very useful in the study of cellular functions of the actin cytoskeleton. For example, phalloidin, which is commonly used for immunofluorescence, binds and stabilizes F-actin (Cooper, 1987). Substances such as latrunculin A and cytochalasin D, on the other hand, promote depolymerization of the filaments, either by forming a complex with actin monomers or by blocking the *barbed end* of the filament through their attachment. Both the growth and branching of F-actin are precisely regulated by several actin binding proteins (Revenu et al., 2004). Capping proteins bind filament ends and thereby vary the length of the filament (e.g., tropomodulin binds and blocks the minus end) by promoting depolymerization (e.g., cofilin binds G-actin), preventing repolymerization (e.g., gelsolin binds to the plus end), or promoting polymerization (e.g., profilin catalyzes the exchange of actin-bound ADP to ATP) (Paavilainen et al., 2004). Other actin-binding proteins, such as filamin, generate flexible actin gels by linking multiple filaments (van der Flier and Sonnenberg, 2001). An important role in cross-linking F-actin is played by the Arp2/3 complex, which binds laterally to an existing filament and serves as a starting point for the polymerization of another filament at a 70° angle (Krause and Gautreau, 2014). Parallel actin fibers, in comparison, are formed into rigid bundles by binding proteins such as α-actinin or fascin (Sjoblom et al., 2008). Over 50 classes of different actin-binding proteins are now known (Edwards et al., 2014). The dynamic structure of actin filaments is regulated by a large number of factors and can, therefore, be quickly adapted to respective cellular needs (Tseng et al., 2005). As a result, some actin structures are the same in all cell types, while others fulfill a very specific function only in individual cell types. In tissues, for example, actin structures are responsible for the polarity of the cells and the cohesion of the epithelial cells or serve as mechanical support for microvilli on the cell surface. During cell division, actin is used in the form of contractile rings to cut off daughter cells from each other. Apart from the contractile apparatus in muscle cells, the actin cytoskeleton plays a major role in cell movement. The assembly and disassembly of actin regulate filopodia and lamellipodia at the cell front of migrating cells, and forces are generated by ATP hydrolysis of the myosin motor proteins at actin fibers.

As the name suggests, microtubules form a hollow, tubelike structure, with a diameter of approx. 25 nm, consisting of 12–17 laterally attached protofilaments. The protofilaments are composed of dimers, which, in turn, are formed of globular α- and β-tubulins. Microtubules originate from the centrosome and grow by polymerization at the plus end toward the cell periphery. During mitosis, they form the spindle apparatus through which the chromosomes are distributed in the daughter cells. In addition, the transport of organelles or vesicles along the microtubules takes place with the help of motor proteins (kinesin, dynein, etc.). The microtubules are among the most rigid elements in animal cells and contribute to the cell's resistance to shear forces through their structural design (Nogales, 2000).

IFs are flexible, stable, and durable protein fibers with a diameter of 10–12 nm, and, in contrast to the other fiber types, do not exhibit polarity. They additionally connect actin filaments and microtubules with each other, whereby their main purpose is the support function; and through the associated protofilaments, the IFs offer high tensile strength. Therefore, they are mainly found in areas of high mechanical force, such as epithelial cells and long-living structures such as hair. They also line the inner nuclear envelope and stabilize the axons of nerve cells. IFs comprise a heterogeneous group of proteins as the fibers are composed of different proteins, depending on the cell type. A distinction is made between type 1 IF made of acidic and type 2 IF made of basic keratins in epithelial cells, and type 3 IF made of vimentin in mesenchymal cells or desmin in muscle cells. Type 4 IF are the neurofilaments of nerve cells, and type 5 IF are the lamins of the cell nuclear envelope (Herrmann et al., 2007).

4.2.2 Integrins: Adhesion Receptor for the Cytoskeleton

Integrins belong to a family of transmembrane glycoproteins and are each composed of an α-subunit and a β-subunit. In vertebrates, 24 different αβ-heterodimers are found, consisting of one of 18 known α- and one of 8 β-subunits (Hynes, 2002). Figure 4.2.2 gives an overview of the possible combinations of α- and β-subunits and their ligands. Each subunit of the heterodimer has a large extracellular, single transmembrane and small intracellular domain (except for β_4-integrin).

The possible combinations of the two extracellular domains specify ligand binding; these are primarily extracellular matrix proteins. Despite the presence of large ligand proteins such as collagen, laminin, and fibronectin, many integrins recognize only short peptide sequences, such as the three amino acids RGD (Arg–Gly–Asp) found in fibronectin and vitronectin. While some integrins recognize only one specific protein (e.g., $\alpha_5\beta_1$ as fibronectin receptor), others have a variety of different binding partners (e.g., $\alpha_v\beta_3$ with laminin, collagen, fibronectin, von Willebrand factor, and fibrinogen) (Kuhn and Eble, 1994). In addition to the expression of different integrin subunits, the specificity can be further increased by alternative splicing of the cytoplasmic domains; thus, the intracellular function of the integrins can be adapted to the respective tissue (Aplin et al., 1998). Both cytoplasmic domains fulfill important tasks with regard to cytoskeletal attachment and signal transduction. Conserved sequences near the plasma membrane keep the two subunits together and in an inactive state, presumably via salt bridges (Wegener and Campbell, 2008). Ligand binding in the cytoplasm (*inside-out* signaling) or from the extracellular domains (*outside-in* signaling) can cause a conformational change so that the two subunits swing apart, and the heterodimer is activated. In this process, the angled, closed conformation of the extracellular domain changes to an upright, open form (Xiong et al., 2001). Signal

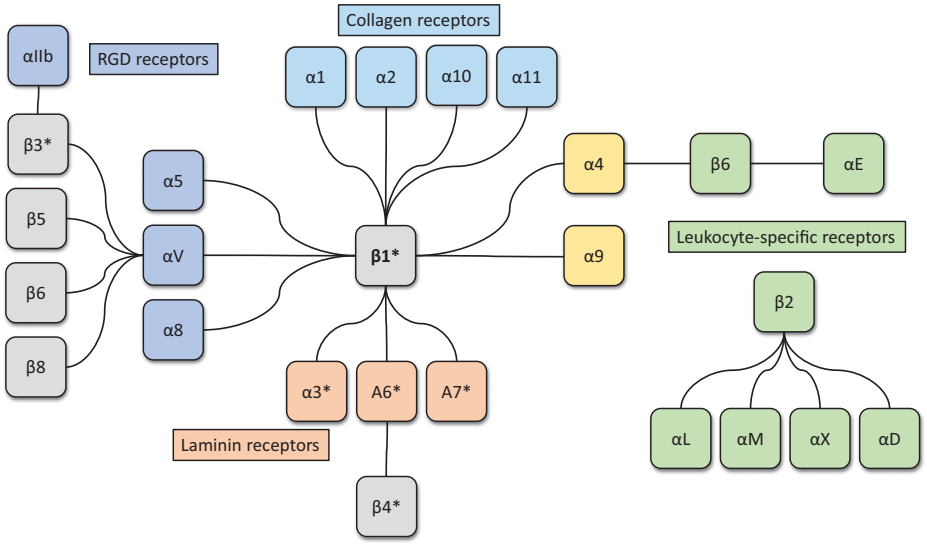

Figure 4.2.2: The integrin family with the various combinations of α- and β-subunits. Large ligand diversity is shown by the frequently occurring integrin heterodimers with β_1- and β_3-subunits. They form receptors for the RGD sequence in fibronectin and vitronectin. β_1Dimers also connect to collagen and laminin. In the basement membrane, $\alpha_6\beta_4$ integrins couple the laminin to intermediate filaments, and heterodimers with β_2- or β_7-subunits are found in cell–cell adhesions of leukocytes. Drawn by Lovis Schween (MSc); Information taken from Hynes (2002).

transduction by integrin molecules can occur in both directions across the plasma membrane. Activation by extracellular ligands often leads to a conformational change that allows cytoplasmic proteins to bind to the intracellular part of the transmembrane proteins, triggering local restructuring of the actin cytoskeleton or activating signaling cascades (Campbell and Humphries, 2011). In contrast, when integrin heterodimers are activated by the interaction of cytoplasmic proteins (e.g., talin), the conformational change of the extracellular domains stimulate binding to matrix proteins and "*clustering*," that is, a local accumulation of integrin molecules in the membrane can occur. This opens up binding sites for extracellular ligands and increases cell adhesion (Liddington and Ginsberg, 2002). Clustering is supported by the lateral homo-oligomerization of the activated α- and β-subunits (Li et al., 2003).

4.2.3 Integrin-Associated Focal Protein Complex

To fulfill the function of chemical and mechanical signal transmission in focal adhesions, the integrins are linked to a multimolecular protein complex on the intracellular side (Calderwood et al., 2003). Over 50 different proteins have already been identified in focal adhesions, which is partly due to cell-specific integrin interactions and partly

due to the complexity of the control processes of these numerous proteins (Bershadsky et al., 2003) (Figure 4.2.3).

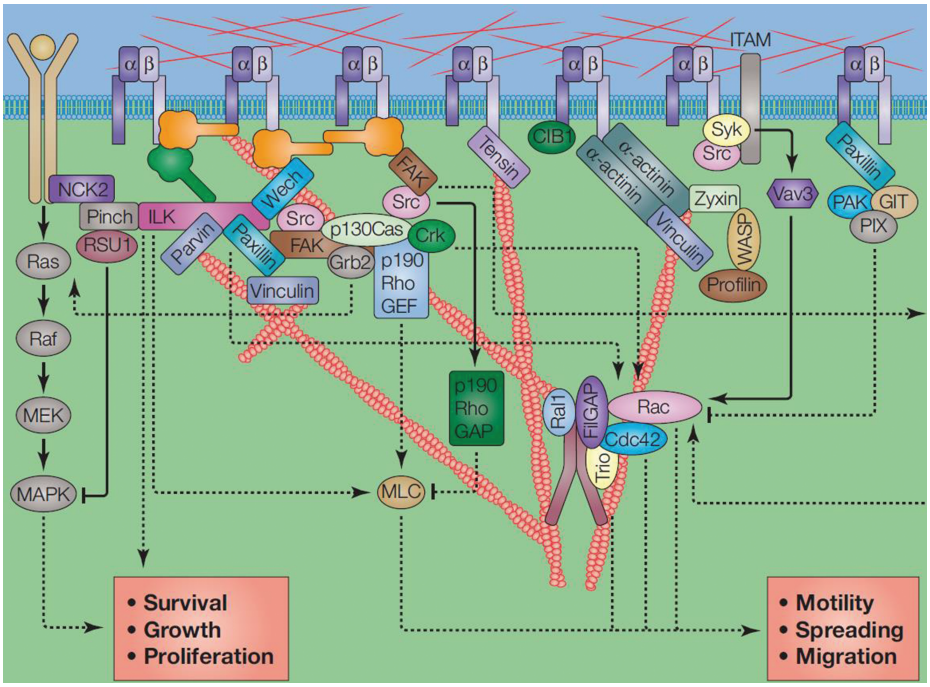

Figure 4.2.3: Proteins involved in the assembly and function of focal adhesions. Through their cytoplasmic domain, integrin heterodimers bind to proteins, such as talin (orange), which, in turn, interact with other focal adhesion proteins (e.g., FAK and vinculin). The entire protein complex then interacts with the actin network. The focal adhesions regulate the actin network via mechanical and biochemical signaling cascades to control the morphodynamics and gene expression of the cell. Taken from Harburger and Calderwood (2009) with permission.

The obligatory cytoplasmic focal contact proteins include talin, paxillin, vinculin, FAK, p130Cas, and α-actinin. In this process, individual proteins such as talin and α-actinin bind directly to integrins and link to other focal proteins such as paxillin and vinculin, ultimately resulting in the recruitment of actin filaments (Brakebusch and Fässler, 2003). The linkage of ECM proteins to the actin cytoskeleton via the integrins and the focal adhesion complex enable bidirectional force transmission (Hynes, 2002). Talin is one of the first proteins involved in the formation of focal contacts and can initiate the activation of integrins. It consists of two polypeptides that form an antiparallel homodimer (Rees et al., 1990). With the N-terminal head domain, talin binds to β_1- or β_3-integrins, as well as to focal adhesion proteins such as FAK or PIP2 (Seelig et al., 2000). PIP2-dependent binding of the FERM domain of talin to an NPXY motif of the β-subunit is a critical step in integrin activation (Nayal et al., 2004). Meanwhile,

the larger domain at the C-terminus of talin interacts with F-actin, as well as with other cytoplasmic binding partners, such as vinculin (Bass et al., 1999). For vinculin, there are three known binding sites (VBS) in the talin protein (VBS1: AS 498–636; VBS2: AS 727–965; VBS3: AS 1943–2157), all of which associate with the same region in the vinculin head domain. Vinculin stabilizes the binding of talin to the actin cytoskeleton, providing a direct mechanical coupling of the force-generating apparatus to the integrins (Giannone et al., 2003). Auernheimer et al. (2015) examined the structure and function of vinculin in focal adhesion protein. Calpain-induced proteolysis of talin can restore the connection between integrins and actin fibers and promotes the dissociation of focal adhesions. Like talin, α-actinin also binds to integrins as well as to actin filaments, thus fulfilling a force-transmitting function (Otey and Carpen, 2004). The actin-bundling protein localizes mainly in mature focal adhesions at the attachment site of contractile stress fibers. That integrins not only serve for attachment to the substrate but are significantly involved in signal transduction is shown, for example, by the integrin-specific increase in phosphorylated proteins in cells adhering to the fibronectin-coated surface. In the focal adhesions, in addition to the stabilizing adapter proteins, numerous proteins involved in signaling are found, such as paxillin, FAK, and p130Cas (Schlaepfer and Hunter, 1998). Phosphorylation (MAP kinases, PKC, Src, FAK) and the concomitant recruitment of paxillin to the focal adhesion complex, in turn, activate additional groups of signaling proteins (Brown and Turner, 2004). As a result, Rho-GTPases are mobilized, and the actin cytoskeleton is reorganized. RhoA, in particular, regulates myosin II activity, whereupon, the motor protein, together with actin filaments, can generate intracellular contractile forces in response to mechanical stimuli (Chrzanowska-Wodnicka and Burridge, 1996). Signaling proteins such as the GTPases Rho and Rac also regulate the kinases that control phosphorylation and thus the function of various focal adhesion proteins. When considering a large number of proteins involved and their different tasks, which are as yet poorly understood and may vary from cell type to cell type, it becomes obvious that focal adhesions are dynamic structures with changing size and composition. Due to mechanical coupling and signaling, focal adhesions regulate the structure of the cytoskeleton, mechanotransduction, migration, proliferation, differentiation, and apoptosis of the cell (Goldmann, 2014).

4.2.4 Phosphorylation

Reversible phosphorylation of proteins is one of the most important post-translational modifications and the most common mechanism for regulating protein function and signal transduction. Approximately one-third of the human proteome is phosphorylated at any one time, and it contains an estimated 500 kinases (Manning et al., 2002). Protein kinases are enzymes that catalyze the transfer of the terminal phosphate

group from adenosine triphosphate (ATP) to the hydroxyl group of one of the amino acids: serine, threonine, or tyrosine. The opposite reaction, that is, the hydrolysis and release of phosphate, is carried out by protein phosphatases. Since kinases recognize not only the target amino acid of their substrate but also the surrounding consensus sequence, some kinases act very specifically on individual proteins, while others phosphorylate multiple substrates (Pawson and Nash, 2003). The effect of phosphorylation on the respective protein is very diverse; for example, the three-dimensional protein conformation can be changed, an enzyme activity can be regulated, or the interaction of proteins with each other can be enabled. Tyrosine kinases are important components of cell proliferation, differentiation, and migration. Many signal transduction cascades rely on the recruitment of cytoplasmic proteins to the membrane, where they bind to phosphorylated receptors or become phosphorylated, themselves. The class of receptor tyrosine kinases (e.g., EGF or insulin receptors) has a transmembrane domain with an extracellular ligand-binding site (receptor) and the intracellular catalytic center (tyrosine kinase). Receptor kinases are activated by ligand binding; they form dimers and can stabilize their active form by autophosphorylation of cytoplasmic tyrosines as well as providing binding sites for other proteins in the signaling chain. The recruited proteins have conserved binding domains for specific amino acids. For example, the domains, SH2 (Src homology 2) and PTB (phosphotyrosine binding) recognize specific phosphotyrosine motifs (pY). Tyrosine kinases without an extracellular ligand-binding receptor domain include the Src, Abl, and FAK kinase families. These cytoplasmic kinases are activated by hormones, neurotransmitters, cytokines, or growth factors. This activation often begins with the phosphorylation of a tyrosine residue. The c-Src kinase is one of nine members of the Src family, which is found in many different cell types and different cell areas. The protein structure of Src kinases, for example, comprises four domains: a catalytic domain SH1, a SH2, and a SH3 domain, a N-terminal membrane localization sequence with a myristic acid residue, and a subsequent specific region for the respective kinase (Boggon and Eck, 2004). Also important is the short C-terminal tail of Src kinase, with the tyrosine residue it contains at position 527. The kinase can be regulated by multiple extracellular signals, including ECM-integrin contacts and, for example, growth factors such as EGF (Parsons and Parsons, 1997). Transient activation occurs through a conformational change by releasing the intramolecular binding of the SH2 domain to pY527 in the C-terminus and exposing the kinase domain. In addition, autophosphorylation of Y416 in the kinase domain is required to achieve full functionality. During the adhesion of fibroblasts to fibronectin, c-Src is dephosphorylated and localizes in focal adhesions (Kaplan et al., 1994). The binding of the SH2 and SH3 domains to p130CAS might play a role in localization or stabilize the open, active conformation of c-Src. Through the same domains, Src kinase can also bind phosphorylated paxillin or focal adhesion kinase. The tyrosine kinase FAK (focal adhesion kinase) is a 125 kDa protein with a central kinase domain and two proline-rich sequences in the C-terminus. Through the FAT sequence, FAK localizes to focal adhesions (Polte and Hanks, 1995). In adherent

cells, integrin signaling and the presence of Src kinase cause an increase in phosphorylated tyrosines in the FAK protein, resulting in increased activity of the kinase, whereas, in detached cells, the protein is again dephosphorylated (Calalb et al., 1995). Src kinase binds to the autophosphorylated FAK protein and thereby, in turn, promotes the association of the adaptor protein p130Cas into the complex, as well as its phosphorylation by FAK (Schlaepfer et al., 1997). The extent of phosphorylation regulates various interactions of FAK, which, in addition to p130Cas, paxillin and talin, binds to a variety of proteins containing an SH2 or SH3 domain (Chen et al., 1995). Thus, FAK also functions as a cross-linking binding partner in the assembly of focal adhesions.

It is clear that the focal adhesion components Src, FAK, p130Cas, and paxillin form a functional unit and are essential for the structure and signaling in adhesions. The activation of tyrosine kinases represents a crucial process of the integrin-mediated signaling cascade, even though it is still unclear how their activation actually occurs. Src, FAK, and other kinases, as well as the antagonistic phosphatases, play an important role in numerous cellular processes, that is, cell growth, migration, apoptosis, gene transcription, the immune response, or neuronal development (Burke, 1994). Reversible phosphorylation, thus, transmits and amplifies signals, so that the cell is able to respond quickly to intra- or extracellular stimuli.

4.2.5 Dynamics and Force Generation via Focal Adhesions

Reversible phosphorylation of proteins is one of the most important post-translational modifications, and the most common mechanism for regulating dynamic cell movement takes place not only in the course of embryogenesis but also in the adult organism, within the tissues. Migration is particularly evident in wound healing, when fibroblasts migrate in, or in metastasis, when individual cells migrate out of the primary tumor and resettle in another part of the body. Although cells are in vivo surrounded by a three-dimensional network of ECM proteins that strongly influence their migration behavior, the basic processes of adhesion and cytoskeleton dynamics can be studied well on two-dimensional substrates. Only the coordinated interplay of force generation and force transmission to the substrate enables the movement and, thus, the response of the cell to external stimuli. For a cell to migrate, it must first adopt a polarized shape, which determines the direction of migration (Figure 4.2.4).

From the actin network at the cell front, broad lamellipodia or single filopodia with long parallel actin fiber bundles are projected toward the membrane by polymerization (Pollard and Borisy, 2003). By protrusion, that is, pushing the membrane forward by local actin polymerization, the cell opens up new territory. The assembly and disassembly of filopodia take place within a few minutes. In order to stabilize a formed

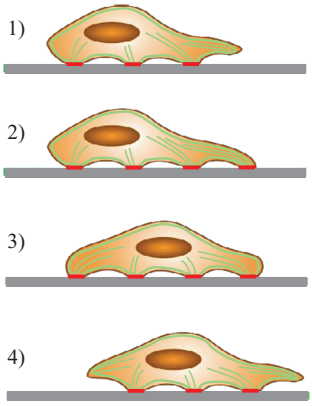

Figure 4.2.4: Four stages of cell migration. (1) Actin polymerization (green) causes the cell to form dynamic protrusions. (2) Certain protrusions are anchored to the substrate by new focal contacts (red). (3) By contraction of the actin cytoskeleton the focal contacts are stabilized. (4) After detachment of existing focal adhesions in the posterior part of the cell, contraction shifts the cell body toward the new adhesions. Taken from commons.wikimedia.org with permission.

filopodium, the actin filaments must be anchored to the extracellular matrix via focal contacts. Tensile forces are established via these new anchors in the cell front by actin-myosin contraction (controlled by Rho kinases) (Beningo et al., 2001). The forces transmitted to the substrate stabilize the new focal adhesions and place the cell under tension (Pasapera et al., 2010). To transform the contraction forces into an efficient forward movement, the adhesions in the posterior part of the cell must detach from the substrate. Once the adhesive structures in the cell rear end have been dissolved (mechanically or biochemically), the cell body moves toward the cell according to the traction forces. Consequently, the spatial distribution of the protrusions and adhesions of different strengths defines the direction of migration of a cell. It is known that cells migrate along gradients of chemical or structure-bound signaling substances (e.g., chemotaxis, haptotaxis). Consequently, signal transduction of the external stimuli and translation into coordinated control of the contractile and adhesive structures must take place. The adhesion process begins with small, punctate, highly dynamic attachments to the substrate, in which, initially, talin establishes a connection between the integrins and the actin filaments (Möhl et al., 2009). This early stage in the cell periphery is also referred to as nascent focal contact. Focal contacts are thought to play a role in the mechanical sensing of the cell as it senses stiffness, geometry, and its environment (Discher et al., 2005, Vogel and Sheetz, 2006, Geiger et al., 2009); their number and size are highly dependent on the properties of the substrate.

Many of these early contacts dissolve within a short time, while others mature into so-called *focal adhesions* through the recruitment of further proteins and the bundling of actin fibers into stress fibers and accompanying force generation (Riveline et al., 2001). The applied forces from the environment, as well as the tensile forces exerted by the actin cytoskeleton from inside the cell, cause a locally enhanced accumulation of integrins in the membrane (clustering) (Choquet et al., 1997) and the accumulation of further proteins, especially vinculin, in the focal complex (Galbraith et al., 2002). In this way, the junction is further stabilized, and there is a growth in the size of the focal adhesions (Golji et al., 2011). In addition to the composition of

the protein complex, the degree of phosphorylation of the proteins also changes. This means that kinases are among the first recruited or activated proteins in the complex (Obergfell et al., 2002). Phosphorylation and dephosphorylation can control the dynamics and maturation stage of focal adhesions (Lele et al., 2008). It is conceivable that an increase in dynamics may lead to destabilization and, in combination with the applied actin traction forces, eventually to the dissolution of the focal adhesion (Wolfenson et al., 2011). The dissociation of focal adhesions must be regulated by diverse signaling pathways in addition to the force exerted, which are thought to involve diverse GTPases, FAK, and also Src kinases (Carragher and Frame, 2004). The tensile forces of the cytoskeleton arise from the interaction of myosin motor proteins and actin filaments. Myosin II induces contraction forces through the lateral displacement of actin fibers relative to each other, similar to the sarcomere of muscle cells.

In general, mechanical signaling pathways rely on a signal being transmitted to biomolecules in the form of mechanical forces, such as tensile forces or shear stress. Often, the applied force induces a conformational change in the protein, exposing functional domains (Del Rio et al., 2009). This can be the trigger for cytoskeletal remodeling, cell shape, or modified gene expression (Chiquet et al., 2009). Mechanical stimuli are often transmitted more rapidly than is the case with the perception of chemical signals (Na et al., 2008). As another example, mechanical traction forces acting externally on the cell have been observed to cause calcium influx across the membrane, which, in turn, causes intracellular force generation and protein recruitment to focal adhesions. Stabilization of focal adhesions and force transmission to the substrate are significantly regulated by proteins such as vinculin (Gallant et al., 2005). Consequently, it is essential to decipher the regulatory mechanisms of vinculin recruitment to understand the signaling pathways and control of focal adhesion formation and dynamics (Goldmann et al., 2013).

4.2.6 Conclusions

Adherent cells are in contact with the extracellular matrix via focal adhesions, a connection that is crucial for many cellular processes. To understand how cells perceive their environment and respond to different stimuli, it is essential to learn more about the regulation and functioning of focal adhesions and the proteins involved. Proteins such as vinculin and talin play a central role in the assembly and disassembly of focal complexes; they stabilize the binding of transmembrane integrins to the actin cytoskeleton of the cell and are, thus, crucial in cellular force transmission. Although intensive research has been conducted for years on focal adhesion proteins and many details about the protein structure and interaction partners are now known, it is still unclear exactly how the activation of the molecules and, thus, their exact function are regulated.

References

Aplin, A. E., A. Howe, S. K. Alahari and R. L. Juliano (1998). "Signal transduction and signal modulation by cell adhesion receptors: The role of integrins, cadherins, immunoglobulin- cell adhesion molecules, and selectins." Pharmacological Reviews **50**: 197–263.

Auernheimer, V., L. A. Lautscham, M. Leidenberger, O. Friedrich, B. Kappes, B. Fabry and W. H. Goldmann (2015). "Vinculin phosphorylation at residues Y100 and Y1065 is required for cellular force transmission." Journal of Cell Science **128**: 3435–3443.

Bass, M. D., B. J. Smith, S. Prigent and D. R. Critchley (1999). "Talin contains three similar vinculin binding-sites predicted to form an amphipathic helix." The Biochemical Journal **341**: 257–263.

Beningo, K. A., M. Dembo, I. Kaverina, J. V. Small and Y. L. Wang (2001). "Nascent focal adhesions are responsible for the generation of strong propulsive forces in migrating fibroblasts." The Journal of Cell Biology **153**: 881–888.

Bershadsky, A. D., N. Q. Balaban and B. Geiger (2003). "Adhesion-dependent cell mechanosensitivity." Annual Review of Cell and Developmental Biology **19**: 677–695.

Boggon, T. J. and M. J. Eck (2004). "Structure and regulation of Src family kinases." Oncogene **23**: 7918–7927.

Brakebusch, C. and R. Fässler (2003). "The integrin-actin connection, an eternal love affair." The EMBO Journal **22**: 2324–2333.

Brown, M. C. and C. E. Turner (2004). "Paxillin: Adapting to change." Physiological Reviews **84**: 1315–1339.

Burke, T. R. Jr. (1994). "Protein-tyrosine kinases: Potential targets for anticancer drug development." Stem Cells **12**: 1–6.

Calalb, M. B., T. R. Polte and S. K. Hanks (1995). "Tyrosine phosphorylation of focal adhesion kinase at sites in the catalytic domain regulates kinase activity: A role for Src family kinases." Molecular and Cellular Biology **15**: 954–963.

Calderwood, D. A., Y. Fujioka, J. M. de Pereda, B. Garcia-Alvarez, T. Nakamoto, B. Margolis, C. J. McGlade, R. C. Liddington and M. H. Ginsberg (2003). "Integrin beta cytoplasmic domain interactions with phosphotyrosine-binding domains: A structural prototype for diversity in integrin signaling." Proceedings of the National Academy of Sciences **100**: 2272–2277.

Carlier, M. F. and D. Pantaloni (1997). "Control of actin dynamics in cell motility." Journal of Molecular Biology **269**: 459–467.

Carragher, N. O. and M. C. Frame (2004). "Focal adhesion and actin dynamics: A place where kinases and proteases meet to promote invasion." Trends in Cell Biology **14**: 241–249.

Carraway, K. L. and C. A. C. Carraway (2000). Cytoskeleton: Signaling and cell regulation. A Practical approach. Hames, B. D., ed. 1st edition, Oxford. UK, Oxford University Press, 1–287.

Campbell, I. D. and M. J. Humphries (2011). "Integrin structure, activation, and interactions." Cold Spring Harbor Perspective Biology **3**: a004994.

Chen, H. C., P. A. Appeddu, J. T. Parsons, J. D. Hildebrand, M. D. Schaller and J. L. Guan (1995). "Interaction of focal adhesion kinase with cytoskeletal protein talin." The Journal of Biological Chemistry **270**: 16995–16999.

Chiquet, M., L. Gelman, R. Lutz and S. Maier (2009). "From mechanotransduction to extracellular matrix gene expression in fibroblasts." Biochimica Et Biophysica Acta **1793**: 911–920.

Choquet, D., D. P. Felsenfeld and M. P. Sheetz (1997). "Extracellular matrix rigidity causes strengthening of integrin-cytoskeleton linkages." Cell **88**: 39–48.

Chrzanowska-Wodnicka, M. and K. Burridge (1996). "Rho-stimulated contractility drives the formation of stress fibers and focal adhesions." The Journal of Cell Biology **133**: 1403–1415.

Cooper, J. A. (1987). "Effects of cytochalasin and phalloidin on actin." Journal of Cell Biology **105**: 1473–1478.

Del Rio, A., R. Perez-Jimenez, R. Liu, P. Roca-Cusachs, J. M. Fernandez and M. P. Sheetz (2009). "Stretching single talin rod molecules activates vinculin binding." Science **323**: 638–641.
Discher, D. E., P. Janmey and Y. L. Wang (2005). "Tissue cells feel and respond to the stiffness of their substrate." Science **310**: 1139–1143.
Edwards, M., A. Zwolak, D. A. Schafer, D. Sept, R. Dominguez and J. A. Cooper (2014). "Capping protein regulators fine-tune actin assembly dynamics." Nature Reviews. Molecular Cell Biology **15**: 677–689.
Galbraith, C. G., K. M. Yamada and M. P. Sheetz (2002). "The relationship between force and focal complex development." The Journal of Cell Biology **159**: 695–705.
Gallant, N. D., K. E. Michael and A. J. Garcia (2005). "Cell adhesion strengthening: Contributions of adhesive area, integrin binding, and focal adhesion assembly." Molecular and Cellular Biology **16**: 4329–4340.
Geiger, B., J. P. Spatz and A. D. Bershadsky (2009). "Environmental sensing through focal adhesions." Nature Reviews. Molecular Cell Biology **10**: 21–33.
Giannone, G., G. Jiang, D. H. Sutton, D. R. Critchley and M. P. Sheetz (2003). "Talin-1 is critical for force dependent reinforcement of initial integrin-cytoskeleton bonds but not tyrosine kinase activation." The Journal of Cell Biology **163**: 409–441.
Goldmann, W. H. (2014). "Mechanosensation: A basic cellular process." Progress in Molecular Biology and Translational Science **126**: 75–102.
Goldmann, W. H., V. Auernheimer, I. Thievessen and B. Fabry (2013). "Vinculin, cell mechanics and tumor cell invasion." Cell Biology International **37**: 397–405.
Golji, J., J. Lam and M. R. Mofrad (2011). "Vinculin activation is necessary for complete talin binding." Biophysical Journal **100**: 332–340.
Harburger, S. D. and D. A. Calderwood (2009). "Integrin signaling at a glance." The Journal of Cell Biology **122**: 159–163.
Herrmann, H., H. Bär, L. Kreplak, S. V. Strelkow and U. Aebi (2007). "Intermediate filaments: From cell architecture to nanomechanics." Nature Reviews. Molecular Cell Biology **8**: 562–573.
Hynes, R. O. (2002). "Integrins: Bidirectional, allosteric signaling machines." Cell **110**: 673–687.
Kaplan, K. B., K. B. Bibbins, J. R. Swedlow, M. Arnaud, D. O. Morgan and H. E. Varmus (1994). "Association of the amino-terminal half of c-Src with focal adhesions alters their properties and is regulated by phosphorylation of tyrosine 527." The EMBO Journal **13**: 4745–4756.
Krause, M. and A. Gautreau (2014). "Steering cell migration: Lamellipodium dynamics and the regulation of directional persistence." Nature Reviews. Molecular Cell Biology **15**: 577–590.
Kuhn, K. and J. Eble (1994). "The structural bases of integrin-ligand interactions." Trends in Cell Biology **4**: 256–261.
Lele, T. P., C. K. Thodeti, J. Pendse and D. E. Ingber (2008). "Investigating complexity of protein-protein interactions in focal adhesions." Biochemical and Biophysical Research Communications **369**: 929–934.
Li, R., N. Mitra, H. Gratkowski, G. Vilaire, R. Litvinov, C. Nagasami, J. W. Weisel, J. D. Lear, W. F. DeGrado and J. S. Bennett (2003). "Activation of integrin alphaII beta3 by modulation of transmembrane helix associations." Science **300**: 795–798.
Liddington, R. C. and M. H. Ginsberg (2002). "Integrin activation takes shape." The Journal of Cell Biology **158**: 833–839.
Manning, G., D. B. Whyte, R. Martinez, T. Hunter and S. Sudarsanam (2002). "The protein kinase complement of the human genome." Science **298**: 1912–1934.
Möhl, C., N. Kirchgessner, C. Schäfer, K. Küpper, S. Born, G. Diez, W. H. Goldmann, R. Merkel and B. Hoffmann (2009). "Becoming stable and strong: The interplay between vinculin exchange dynamics and adhesion strength during adhesion site maturation." Cell Mot Cytoskeleton **66**: 350–364.

Na, S., O. Collin, F. Chowdhury, B. Tay, M. Ouyang, Y. Wang and N. Wang (2008). "Rapid signal transduction in living cells is a unique feature of mechanotransduction." Proceedings of the National Academy of Sciences **105**: 6626–6631.

Nayal, A., D. J. Webb and A. F. Horwitz (2004). "Talin: An emerging focal point of adhesion dynamics." Current Opinion in Cell Biology **16**: 94–98.

Nogales, E. (2000). "Microtubule function." Annual Review of Biochemistry **69**: 277–302.

Obergfell, A., K. Eto, A. Mocsai, C. Buensuceso, S. L. Moores, J. S. Brugge, C. A. Lowell and S. J. Shattil (2002). "Coordinate interactions of Csk, Src, and Syk kinases with alphaIIb beta3 initiate integrin signaling to the cytoskeleton." The Journal of Cell Biology **157**: 265–275.

Otey, C. A. and O. Carpen (2004). "Alpha-actinin revisited: A fresh look at an old player." Cell Mot Cytoskeleton **58**: 104–111.

Paavilainen, V. O., E. Bertling, S. Falck and P. Lappalainen (2004). "Regulation of cytoskeletal dynamics by actin-monomer-binding proteins." Trends in Cell Biology **14**: 386–394.

Parsons, J. T. and S. J. Parsons (1997). "Src family protein tyrosine kinases: Cooperating with growth factor and adhesion signaling pathways." Current Opinion in Cell Biology **9**: 187–192.

Pasapera, A. M., I. C. Schneider, E. Rericha, D. D. Schlaepfer and C. M. Waterman (2010). "Myosin II activity regulates vinculin recruitment to focal adhesions through FAK-mediated paxillin phosphorylation." The Journal of Cell Biology **188**: 877–890.

Pawson, T. and P. Nash (2003). "Assembly of cell regulatory systems through protein interaction domains." Science **300**: 445–452.

Pollard, T. D. and M. S. Mooseker (1981). "Direct measurement of actin polymerization rate constants by electron microscopy of actin filaments nucleated by isolated microvillus cores." The Journal of Cell Biology **88**: 654–659.

Pollard, T. D. and G. G. Borisy (2003). "Cellular motility driven by assembly and disassembly of actin filaments." Cell **112**: 453–465.

Pollard, T. D. and J. A. Cooper (2009). "Actin, a central player in cell shape and movement." Science **326**: 1208–1212.

Polte, T. R. and S. K. Hanks (1995). "Interaction between focal adhesion kinase and Crk associated tyrosine kinase substrate p130Cas." Proceedings of the National Academy of Sciences **92**: 10678–10682.

Rees, D. J., S. E. Ades, S. J. Singer and R. O. Hynes (1990). "Sequence and domain structure of talin." Nature **347**: 685–689.

Revenu, C., R. Athman, S. Robine and D. Louvard (2004). "The co-workers of actin filaments: From cell structures to signals." Nature Reviews. Molecular Cell Biology **5**: 635–646.

Riveline, D., E. Zamir, N. Q. Balaban, U. S. Schwarz, T. Ishizaki, S. Narumiya, Z. Kam, B. Geiger and A. D. Bershadsky (2001). "Focal contacts as mechanosensors: Externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism." The Journal of Cell Biology **153**: 1175–1186.

Schlaepfer, D. D., M. A. Broome and T. Hunter (1997). "Fibronectin-stimulated signaling from a focal adhesion kinase-c-Src complex: Involvement of the Grb2, p130cas, and Nck adaptor proteins." Molecular and Cellular Biology **17**: 1702–1713.

Schlaepfer, D. D. and T. Hunter (1998). "Integrin signaling and tyrosine phosphorylation: Just the FAKs?." Trends in Cell Biology **8**: 151–157.

Seelig, A., X. L. Blatter, A. Frentzel and G. Isenberg (2000). "Phospholipid binding of synthetic talin peptides provides evidence for an intrinsic membrane anchor of talin." The Journal of Biological Chemistry **275**: 17954–17961.

Sjoblom, B., A. Salmazo and K. Djinovic-Carugo (2008). "Alpha-actinin structure and regulation." Cellular and Molecular Life Sciences: CMLS **65**: 2688–2701.

Tseng, Y., T. P. Kole, J. S. Lee, E. Fedorov, S. C. Almo, B. W. Schafer and D. Wirtz (2005). "How actin crosslinking and bundling proteins cooperate to generate an enhanced cell mechanical response." Biochemical and Biophysical Research Communication **334**: 183–192.

van der Flier, A. and A. Sonnenberg (2001). "Structural and functional aspects of filamins." Biochimica et Biophysica Acta **1538**: 99–117.

Vogel, V. and M. P. Sheetz (2006). "Local force and geometry sensing regulate cell functions." Nature Reviews. Molecular Cell Biology **7**: 265–275.

Wegener, K. L. and I. D. Campbell (2008). "Transmembrane and cytoplasmic domains in integrin activation and protein-protein interactions." Molecular Membrane Biology **25**: 376–387.

Wolfenson, H., A. Bershadsky, Y. I. Henis and B. Geiger (2011). "Actomyosin-generated tension controls the molecular kinetics of focal adhesions." Journal of Cell Science **124**: 1425–1432.

Xiong, J. P., T. Stehle, B. Diefenbach, R. Zhang, R. Dunker, D. L. Scott, A. Joachimiak, S. L. Goodman and M. A. Arnaout (2001). "Crystal structure of the extracellular segment of integrin alphaV beta3." Science **294**: 339–345.

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6.8 Intermediate Filaments

Abstract: Intermediate filaments (IFs) are one of the three types of cytoskeletal polymers that resist tensile and compressive forces in cells. They cross-link with each other as well as with actin filaments and microtubules by means of proteins such as desmin, lamin (A/C), plectin, and filamin C. Mutations in these proteins can lead to a wide range of pathologies, some of which exhibit mechanical failure of the skin, skeletal, or heart muscle.

Keywords: cellular mechanics, desmin, filamin C, intermediate filaments, lamin (A/C), plectin

6.8.1 Introduction

Intermediate filaments (IFs) are found in many cell types and are part of the actin filament and microtubule cytoskeleton (Figure 6.8.1). They extend throughout the cytoplasm connecting the nuclear and cell membrane and are responsible for cell morphology and mechanics (Capetanaki et al., 2007, Fletcher and Mullins, 2010, Etienne-Manneville, 2018). While extra-sarcomeric IFs constitute a filamentous network through a number of cross-linking and regulatory proteins in cells that connect membrane-anchored structures with Z-disks, sarcomeric IF proteins integrate the cytoskeleton with organelles such as mitochondria and nuclei. Various IF protein types have been described in many cell types, whose staggered assembly into protofilaments impart high tensile strength, thus enhancing their resistance to compression, stretching, and bending forces (Herrmann et al., 2009, Goldman et al., 2011, Köster et al., 2015, Herrmann and Aebi, 2016, Brennich et al., 2019).

In the following, the four prominent proteins (desmin, lamin (A/C), plectin, and filamin C) from an IF network will be described in terms of their biological, disease, and mechanical function in living cells. Such IF proteins have been reported as important contributors to cellular contractility and prestress and serve as molecular "guy wires" that facilitate the transfer of mechanical loads between the cell surface

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and the nucleus, and thereby stabilize microtubules and actin filaments (Winter and Goldmann, 2015). A complete list of all IF proteins and their characteristics in various cell lines is provided in Cooper (2000).

6.8.2 Desmin and Diseases

Desmin is the most commonly studied disease entities in human myofibrillar myopathies. It belongs to the group of IFs that form 3D extra-sarcomeric filamentous networks in cells and is responsible for a number of functions, including maintenance of sarcomeres, specific positioning of organelles, and cell signaling. Desmin-deficient cells compromise the general organization of muscle fibers in that they misalign and mislocate myofibrils and mislocate nuclei and mitochondria. In certain neurodegenerative diseases, desmin mutations can trigger increased oxidative stress and cause abnormal protein aggregates. Moreover, inhibition of the clearance mechanisms during ageing might exacerbate protein accumulation and contribute to the progression of the disease (Schröder et al., 2007, Schröder and Schoser, 2009, Clemen et al., 2009, 2013, Winter et al., 2019, Herrmann et al., 2020, Spörrer et al., 2022).

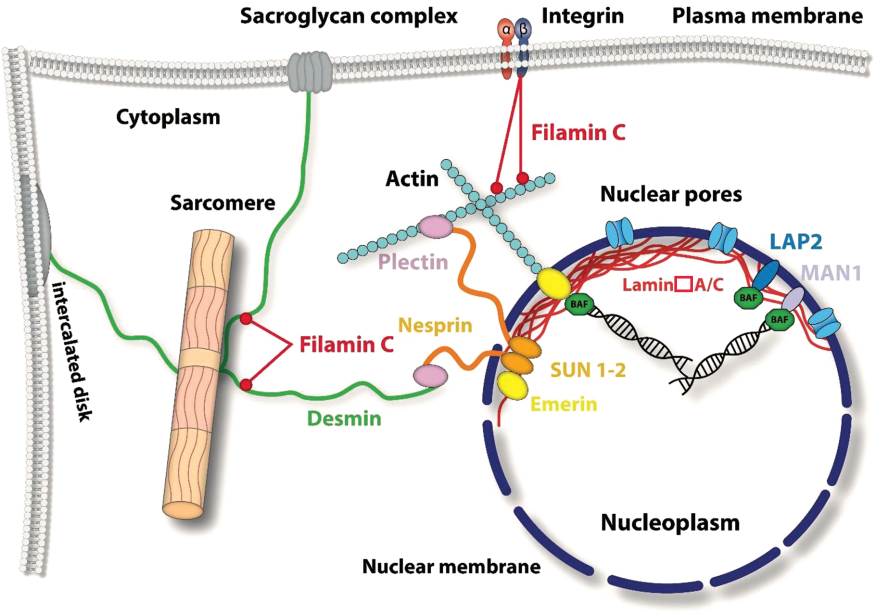

Figure 6.8.1: A representation of the intermediate filament proteins in the cell. These interact with the actin cytoskeleton connecting with integrins, the nuclear membrane, and intercalated disk, forming a tight cellular filament network. The different proteins are color-coded in the graph. Taken from Cell Biol. Int. (2018) **42**: 321 with permission.

The autosomal dominant missense p.Arg350Pro belongs to a subset of desmin mutations as the most commonly reported mutation. The exchange of arginine through proline affects the unfolding during desmin assembly that could lead to IF collapse. To date, the propensity of desmin mutants to modify the normal organization of myogenic cells has only been analyzed in murine C2C12 myoblast clones, expressing exogenously desmin mutants (Charrier et al., 2016). Disease mutations identified more recently showed skeletal and cardiac myopathies that correlate with pathological protein aggregation. Mücke et al. (2016) dissected the pathway and the kinetics of desmin assembly, in detail; and it was shown that its pathway deviates significantly from that of vimentin, another IF protein (Mücke et al., 2018, Schween et al., 2022). Further, comparing the assembly kinetics of mutant and wild-type desmin indicated how the interaction between the plakin family and cellular chaperones influence the assembly.

6.8.3 Desmin and Cell Mechanics

An important question raised within the present research community is the function of desmin and how its mutants exert their deleterious effects on human skeletal and cardiac muscle cells, with respect to their structure and function. Here, desmin is a key component of the 3D filamentous extra-sarcomeric cytoskeleton that interlinks neighboring myofibrils at the Z-disk, connecting the entire myofibrillar apparatus to costameres, intercalated discs, myotendinous, and neuromuscular junctions (Hnia et al., 2015). This network provides important anchorage points for the alignment of myofibrils and for the attachment to the sarcolemma, nuclei, and mitochondria by performing the important function of adapting striated muscle fibers to active and passive stresses. Studies in desminopathic patients showed that heterozygous/homozygous mutations affect the structure and function of the extra-sarcomeric network in different ways; however, nothing is known about the early disease stages that actually precede the clinical manifestation of muscle weakness in human desminopathies. To address this issue, Clemen et al. (2015) used hetero- and homozygous R349P knock-in mice, which possess the ortholog of the most frequently occurring human desmin missense mutation, R350P. The mice exhibited age-dependent skeletal muscle weaknesses, dilated cardiomyopathies, cardiac arrhythmias, and conduction defects. Further, as described in a mouse model, morphological and biomechanical alterations were evident in the early disease stages. Using nonlinear second harmonic generation (SHG) and 2-photon fluorescence morphometry analysis in combination with active and passive biomechanical recordings of muscle fibers, Diermeier et al. (2017a) unveiled an early disease pattern, in which mutant desmin showed aberrant myofibrillar alignment and orientation as the basis for compromised active force production. These authors showed altered

passive and biomechanical properties, which made them more prone to fiber damage and provided initial insights into adaptive mechanisms that may compensate for force discrepancies in preclinical disease. Further, Diermeier et al. (2017b) used small fiber bundles from unfixed soleus mice muscles in multicellular biomechanics experiments. Since the morphological pathology of R349P desmin knock-in mice is most prominent in soleus muscle, these researchers restricted their biomechanical experiments to this mutation.

Fiber bundles were also used in a mechatronic device called "MyoRobot," which was custom-built, is automated, and mimics skeletal muscle (Haug et al., 2019). The fiber bundles, here, were measured by a force transducer pin and software-controlled voice coil actuator. An automated image-processing algorithm developed by Buttgereit et al. (2013) was used for the morphometric analysis of 3D SHG and multiphoton fluorescence images. A second morphometric parameter extracted from SHG microscopy called "verniers" described Y-shaped deviations that resulted from out-of-register deviations in the regular signal pattern of adjacent myofibrils (Friedrich et al., 2019).

These researchers tested the hypothesis that the mutated R349P desmin also exerts a detrimental effect on biomechanics by testing the steady-state axial elasticity of small fiber bundles. Two recordings from Des^{R349P} soleus fiber bundle experiments were carried out with simultaneous measurements on the individual, stretch-related, passive restoration force. Results from quasi-static passive biomechanics showed higher axial elastic stiffness in hetero- and homozygous Des^{R349P} soleus fiber bundles compared to the wildtype (Diermeier et al., 2017b). To determine the viscoelastic behavior of soleus fiber bundles from Des^{R349P} mice, stretch-jump experiments were also performed by stretching bundles successively; however, the relaxation kinetics proved inconsequential among the genotypes.

Subcellular morphological alterations detected by SHG provided a structural basis for explaining early alterations in biomechanical properties of slow-twitch muscle in Des^{R349P} desminopathy (Buttgereit et al., 2013, 2014). Since desmin is also known to link to the nuclear domain and the sarcoglykan complex of muscle fibers (Goldfarb and Dalakas, 2009), an impairment of mutant Des^{R349P} desmin as a means to contribute to lateral compliance was also suggested by Bonakdar et al. (2012) for human Des^{R350P} myoblasts. Further evidence for the effects of the Des^{R350P} mutation on the viscoelastic properties of IF-networks emerged from in vitro bulk assembly studies, where Des^{R350P} exhibited a merely weak increase in viscosity, when assembled on its own, but showed a marked hyperviscosity when co-assembled with equimolar amounts of wildtype desmin (Bär et al., 2006). Interestingly, mutations in the tail domain of desmin highlighted diminished stiffening in filament networks (Bär et al., 2010).

Each of the aforementioned studies provided initial insights into the detailed effects of the murine R349P desmin knock-in mutation on the passive and active biomechanical properties in preclinical stages of skeletal muscle, where desmin-positive protein aggregates are not yet present. The studies are supported by state-of-the-art

multiphoton microscopy data that showed vast morphological alterations in the subcellular architecture of both fast- and slow-twitch muscle fibers, which point toward myofibrillar lattice disruptions that are more evident/accentuated in slow-twitch muscle (soleus). The lattice disruptions and less tightly oriented myofibrils suggest a compromise in biomechanical properties consistently observed in the passive quasi-static elasticity and for viscoelastic properties. Simply speaking, desminopathic muscle fiber bundles, myofibrillar bundles, as well as the membrane complex in myoblasts carrying the very same mutation were much stiffer compared to wildtype desmin. The severity of increased stiffness depended on the maturation level and was more pronounced in homozygous mutations in the preclinical adult stages (fiber and myofibrillar bundles). This might explain why affected muscles are prone to stretch-induced injury and aggravate subsequent protein aggregate formation, which is more pronounced in slow-twitch muscle. Interestingly, homozygous soleus muscle fibers show, by means of a mechanism not yet confirmed, a compensation of force over heterozygous preparations that otherwise reflect reduced myofibrillar Ca^{2+} sensitivity. Since the heterozygous Des^{R350P} genotype in humans is pathologically predominant, as reflected by the murine Des^{R349P} genotype, the specific result fully explains the detrimental effects of a single mutated desmin allele in affected patients: compromised passive extensibility of muscle, cellular architecture, and active force production (Diermeier et al., 2017b).

6.8.4 Lamin A/C and Diseases

Nuclear lamins are cytoskeletal proteins that belong to the family of IFs and are located on the inner nuclear membrane. There are two main classes of lamin proteins, A and B-type. B-type lamins are further classified into B1-lamins and B2-lamins encoded through the genes LMNB1 and LMNB2, respectively. As many mutations and particularly the lack of B-type lamins were found to be lethal to cells, no genetically inheritable disease is connected to mutations in the LMNB genes. In contrast to that, LMNA, the gene that encodes the A-type lamins A, AD10, C, and C2 is one of the most mutated genes in humans. The loss of A-type lamin function, however, can still lead to serious diseases, so-called laminopathies. The most prominent of these diseases are Emery-Dreifuss muscular dystrophy, cardiomyopathies, and premature ageing syndromes like Hutchinson-Gilford progeria syndrome. All the above laminopathies can be correlated to point mutations on the LMNA gene. Thus, the exact mechanism between the nanoscale punctual mutation and macroscopic changes of the tissue of diseased patients is largely unclear. It has repeatedly been suggested that laminopathies stem from a disturbance of a gene-regulating function in the LMNA gene. A general mechanical weakness, likely caused by diminished nucleocytoskeletal integrity

after lamin A-loss, has previously been suggested to cause laminopathy (Bonne et al., 1999, Moir et al., 2000, Vignier et al., 2018, Pfeifer et al., 2019).

The nucleus is the most prominent cell organelle in all eukaryotic cells. It contains most of the cell's genetic material in the form of heterochromatin, euchromatin, and nucleoli, and protects and controls the genetic replication machinery. The nucleus regulates gene expression through various transmembrane nuclear pore complexes and channels and defines cell mechanical properties to a large extent, due to its dominating volume and higher stiffness. The deformability of the cell nucleus may likely be regulated by the state of chromatin, since chromatin condensation correlates with cell stiffness. The cell nucleus is surrounded by the nuclear membrane and stabilized by the nuclear skeleton, also called the nuclear lamina. The lamina is an organized meshwork of IFs, mainly lamin A/C and B, located at the interior boundary of the nuclear membrane, providing support and anchoring points for pores and channels. The nuclear lamina is connected to the cell cytoskeleton, e.g., to actin, microtubule, and IFs through LINC-complexes. These are assembled by the transmembrane proteins, SUN and nesprin, which interact with molecular motors such as dynein (Fatkin et al., 1999, Lloyd et al., 2002, Broers et al., 2004, Brull et al., 2018).

6.8.5 Lamin A/C and Cell Mechanics

IFs are a family of related cytoplasmic and nuclear proteins, which are, on average, 10 nm in diameter. They are categorized into six subfamilies according their similarities to amino acid and protein structures. Prominent examples are keratins (I and II), desmin and vimentin (III), neurofilaments (IV), lamins (V) and nestin (VI) (Mücke et al., 2018). The existence and number of certain IFs greatly depend on the cell type and their function. IFs are the least stiff of the three cytoskeletal proteins, having a Young's modulus of around 4×10^6 (Pa) (Charrier and Janmey, 2016). Moreover, they have a persistence length of only 1 micrometer, but are reported to counterbalance large strains. Lamins were found to play an important role in the cell's protection against nuclear stresses during the migration through confined spaces, thereby the nuclear lamina is assumed to function as protection against DNA compression and shear.

Local force generation, dynamic modification of stiffness, the viscosity of cells, and their responses to traction or compressional forces are general hallmarks of cellular and tissue mechanics (Dahl and Kalinowski, 2011). These parameters were examined by Lee et al. (2007) in lamin A-deficient mouse embryonic fibroblasts (MEFs). Either the disassembly of actin filaments or microtubule networks proved to lead to the decrease in cytoplasmic elasticity and viscosity. Further, studies by Lanzicher et al. (2015), using atomic force microscopy (AFM) on cardiomyocytes, which carry a lamin A/C mutation (D192G), showed increased maximum nuclear deformation load, nuclear stiffness, and fragility compared to control cells. They deduced from their

experiments that a non-association of the cytoskeleton with lamins was the trigger for cellular morphological and adhesive changes that could lead to reported fatal cardiomyopathies (Chatzifrangkeskou et al., 2018). Aptke et al. (2017) investigated the mutation E145K on lamin A, which has been shown to cause Hutchinson-Gilford progeria syndrome (HGPS), by using the atomic force microscope. They found that this mutation dramatically increased nuclear stiffness compared to the wildtype in Xenopus oocytes.

Mechanical studies on lamina A-mutated in vitro systems have been conducted in a wide range of research facilities during the last years (Nikolova et al., 2004, Lammerding et al., 2004, 2006, Osmanagic-Myers et al., 2015b, Mitchell et al., 2015, Kolb et al., 2017). Pivotal results from Lange et al. (2015, 2017) showed that lamin A, in contrast to B-type lamins, is linked to cell mechanosensing, suggesting that lamin A is upregulated on stiffer matrix surroundings. Moreover, lamin A was found to hinder, but at the same time, to protect the cells against nuclear stresses during cell migration through confined spaces. Thereby, the nuclear lamina is assumed to function as protection against DNA compression and shear. Still, several questions remain unclear, one of which is, how the loss of lamin A results in a possible overall cell weakening, where the dose–response of lamin A-loss or overexpression on cell mechanics is concerned. In studies with K562 leukemia cells overexpressing lamin A, Lange et al. (2015) used the microconstriction methods and investigated lamin A fluorescence extension. Depending on the fluorescence expression levels after measurement, cells were sorted accordingly. This can be attributed to the fact that averaging the mechanical properties over the entire population would almost certainly lead to biased results. Such mechanical properties would, in turn, strongly depend on the transfection efficiency during the actual transfection process.

Expression levels of nuclear lamins have also widely been connected with overall cell stiffness and fluidity. All three network components are highly connected to each other, to the nucleus via LINC complexes, and to the cell membrane via focal adhesion sites and integrins. This poses a problem, when investigating the mechanical properties of reconstituted cytoskeletal networks in vitro and applying this knowledge to the in vivo complex system of a cell cytoskeleton.

Summarizing the above, cell mechanical measurements with a microconstriction setup showed that cell stiffness increases significantly in a dose–response manner with lamin A-overexpression level. At the same time, cell fluidity decreases significantly. The reason for this clear-cut correlation may be that lamin A supports the integrity of the nuclear lamina. The nuclear lamina, in turn, is connected to all other cell cytoskeletal components through so-called LINC complexes and might therefore provide stability for the actin cytoskeleton, as well. These results are in accordance with previous measurements on lamin A overexpressing adherent cells and nuclei. To the author's knowledge, a dose–response curve associating lamin A overexpression with cell stiffness and fluidity has not been explored.

Recently, Schürmann et al. (2016) examined the cellular mechanics of human fibrosarcoma (HT1080) cells in 2D under isotropic stretch in cells with overexpressed lamin A. From their results, they assumed stiffening of the nucleus membrane area and the cytoskeleton, as the cell area was smaller in these cells, compared to control cells for stretches up to 10%. The authors showed that, the increased stiffness of the mutant HT1080 cells resulted in complete detachment of cells from the extracellular matrix at 15% stretch, which confirmed the stiffening of the global cellular cytoskeleton through an isolated increase in nuclear stiffness in lamin A overexpressing cells.

To explain how mutations in lamin A of the nuclear envelope can affect the heart muscle, it has been proposed that nuclear envelope abnormalities can cause cellular fragility and decrease the mechanical resistance to stress. This could partially explain hypertrophic cardiac muscle disease, considering that the heart muscle is constantly subjected to mechanical force. It is believed that abnormal activation of stress-activated ERK1/2 signaling in mice hearts that carry lamin A mutations might be the cause. Administering drugs which inhibit ERK1/2 signaling could improve cardiac ejection fraction. Recent observations by Schwartz et al. (2017) also showed that pathogenic LMNA mutations in human muscle precursor cells, which are responsible for severe muscle dystrophies, exhibit accumulated contractile stress fibers, increased focal adhesions, and higher traction force, compared to control cells. Thus, deactivating the ROCK-dependent regulator, formin, responsible for remodeling actin, preserves the morphology of mutant cells. Further, the functional integrity of lamin/nesprin-1 is necessary to modulate formin and cellular mechanical coupling. Previously, the role of cell and nuclear stiffness was investigated on multiple cell lines (the fibrosarcoma cell line HT-1080 and the breast cancer cell line MDA MB-231). These cell lines overexpressed lamin A that migrated through 3D devices consisting of a linear channel with a length of 630 μm, height of 3.7 μm, and decreasing channel width from 11.2 to 1.7 μm (Lautscham et al., 2015). All cell lines showed reduced cell migration, which was attributed to higher cell stiffness and lower adhesiveness. To separate the effect of cell stiffness from other invasion-modulating cell properties, the expression levels of lamin A were increased, which correlated with nuclear stiffness. The authors hypothesized that cells with higher lamin A levels experience higher resistance, when migrating through confined spaces due to the increased cell stiffness. In another study, the effect of lamin A by means of microconstriction method was investigated (Lange et al., 2015). To test how lamin A overexpression affects the overall cell mechanical properties, the stiffness and fluidity of various cells (leukemia cells, K562, and breast cancer cell line, MDA MB-231) were measured. Compared to wildtype cells, the stiffness cells that were overexpressed by lamin A increased significantly (Lange et al., 2017). This data confirms that lamin A contributes greatly to cell stiffness, but the method does not discriminate between the stiffness of the cell nucleus and the cytoskeleton. Lange et al. (2017) were ultimately unable to exclude the possibility that lamin A overexpression leads to altered cytoskeletal mechanics and structure.

More recently, we investigated the impact of A-type lamin (p.H222P) mutation on the mechanical properties of muscle cells by microconstriction rheology. We demonstrated that the expression of point mutation of lamin A in muscle cells increases cellular stiffness compared to cells expressing wild type lamin A, and that the chemical agent selumetinib, an inhibitor of the ERK1/2 signaling, reversed the mechanical alterations in mutated cells. These results highlight the interplay between A-type lamins and mechano-signaling, which are supported by cell biology measurements (Chatzifrangkeskou et al., 2020).

6.8.6 Plectin and Diseases

Plectin was first reported by Wiche et al. (1982), who found that plectin gene defects cause epidermolysis bullosa simplex with muscular dystrophy (EBS-MD). This, in turn, is characterized by severe skin blistering and muscular dystrophy. Using skeletal muscle, Wiche et al. (1982) showed that at least four plectin isoforms are responsible for targeting and linking desmin IF networks to Z-disks, costameres, mitochondria, and the nuclear/ER membrane system, severe skin blistering, and muscular dystrophy. Plectin deficiency leads to desmin aggregation and mitochondrial dysfunction. Further, they established numerous plectin isoform-specific knock-out mouse strains, elucidating the function of plectin in normal and EBS-MD muscles (Andrä et al., 1997). Moreover, Konieczny et al. (2008) established several plectin isoform-specific and conditional knock-out mouse strains, of which two closely mirror the human EBS-MD muscle pathology. Special focus was directed to plectin-mediated effects on the structure and function of the desmin cytoskeleton, mitochondrial positioning, and metabolism, as well as intracellular signaling events, including AMPK-mediated energy homeostasis, the mTOR pathway, and apoptosis.

6.8.7 Plectin and Cell Mechanics

Plectin is a prominent cytoskeletal linking protein based on IFs. It strengthens cells mechanically by interlinking, anchoring cytoskeletal filaments, and acting as scaffolding- and docking platform for signaling proteins. In this function, it controls the dynamics of the cytoskeleton; however, research results of its biomechanical effects in muscle are scarce. Hijikata et al. (1999) showed that plectin links desmin IFs to Z-disks and prevents individual myofibrils from disruptive contractions. Although Na et al. (2009) examined its role in setting cell stiffness, stress propagation, and traction generation in wildtype plectin and plectin-deficient skin fibroblasts, its influence on muscle biomechanics through the various organ scales was not known. Thus, Bonakdar

et al. (2012) showed that pathogenic plectin mutations cause increased cell stiffness due to higher baseline contractile activation. This leads to higher intracellular stress during cyclic stretch and, consequently, to higher stress vulnerability in muscle. In related experiments, Winter et al. (2014) investigated the effect of a plectin knock-out in mouse myoblasts. These experiments are particularly relevant because the same procedure for obtaining immortalized myoblasts with the knock-out mutations of extrasarcomeric cytoskeletal proteins was followed in all subsequent studies. Cell stiffness was decreased two times in the plectin knock-out cells. In agreement with lower stiffness, plectin knock-out cells showed a higher power-law exponent of the creep modulus, indicating a less stable cytoskeleton and a more fluid-like mechanical behavior of these cells. Furthermore, plectin knock-out cells were approximately 2.5 times less contractile, which indicates a diminished cytoskeletal prestress that is likely the primary cause for lower stiffness in these cells (Bonakdar et al., 2015). The hypothesis that cell death after stretching is caused by stretch-induced mechanical stress correlates with cell stiffness. Osmanagic-Myers et al. (2015a) confirmed that the softer plectin knock-out cells are approximately twice as less vulnerable to cyclic stretch, compared to wildtype cells. Almeida et al. (2015) also showed that plectin is an essential regulator of nuclear morphology and protects the nucleus from mechanical deformation.

6.8.8 Filamin C and Diseases

Filamin C is an actin-binding and regulatory protein that is closely associated in myofibril formation. The first mutation in the filamin C gene that caused myofibrillar myopathy (MFM) in humans was reported by Vorgerd et al. (2005). Studying the pathogenic consequences, these authors provided the biochemical evidence for altered filamin C properties that lead to protein aggregation. Further, in-depth studies on the pathogenesis of filamin C myopathy were carried out by the group of Dr. Fürst, who used ES cells stably transfected with wildtype and mutant filamin C as well as human samples (Fürst et al., 2013). More recently, compelling evidence of filamin C's involvement in human hypertrophic cardiomyopathy was shown with the help of SIFT and other screening algorithms (Gomez et al., 2017).

In cells, filamin C binds to both alpha-actinin and actin and can interact with the co-chaperone BAG3 and with the membrane fusion machinery containing the VPS protein (Selcen et al., 2009). These interactions are essential for chaperone-assisted selective autophagy (CASA) and found in muscle. Filamin C isoforms may also have degradation-independent functions in the regulation of mechanical-stress-related signaling pathways, thus necessitating the precise subcellular localization and dynamic behavior of all filamin C protein variants in muscle cells. Functional studies should therefore reveal their involvement in mechanically stress-induced degradation and

signaling. Further, the impact of phosphorylation and the dynamics of complexes containing filamin C protein, in the context of contractile activity, is of importance; a similar study was conducted for desmin (Diermeier et al., 2017a).

6.8.9 Filamin C and Cell Mechanics

Filamin C is a key component of sarcomeric Z-disks and cell–matrix contacts, where it binds to a wide range of cytoskeletal and signaling proteins and to a large number of proteins, including aciculin, Xin, XIRP2, FILIP-1, myotilin, and podins. Further, many phosphorylation sites on filamin C have been identified, which could give the protein a regulatory function. It was shown that mechanical activity directly alters the dynamic behavior of filamin C and its interaction partners, and that protein complexes are immediately recruited to mechanically damaged areas. A lack or the functional impairment of components in this regulatory network has been reported to have severe muscle damage in human patients and animal models.

In our first experiments, we asked whether mechanical stress has a different effect on the dynamics of mutant filamin C than on wildtype cells (Winter and Goldmann, 2015). We were able to show that molecular processes contribute to a reduced mechanical stress resistance in diseased muscle cells, using live-cell confocal microscopy and protein expression studies on myoblasts derived from p.W2710X filamin C knock-in mice. Early, unpublished results have suggested that: (i) filamin C mutant cells detach at a higher percentage compared to wildtype cells after external stress application, (ii) the strain energy of mutant cells is lower compared to wildtype cells, and (iii) the stiffness of mutant cells is higher compared to wildtype cells. Some observations were confirmed by Chevessier et al. (2015), in that mutant filamin C in muscle interferes with the mechanical stability and strain resistance of myofibrillar Z-disks.

However, more in-depth studies are needed to unravel the biomechanical mechanisms responsible for muscle weakness in the filamin C myopathy (p.W2710X), using skeletal muscle preparations (whole muscles, fiber bundles, single fibers, myotubes) from heterozygous and homozygous mice. The role of mutated filamin C affecting the lateral versus the axial biomechanical properties should be elucidated. This can be accomplished using force transducer recordings in single myofibers with intact integrin–filamin C complexes and mechanically skinned fibers after removal of the sarcolemma, thereby leaving only the filamin C anchorage at the Z-disk. The lateral compliance can be determined through magnetic tweezer experiments in myotubes and in intact single fibers among genotypes. As a novel approach, the nonlinear tractions of the integrin–filamin C complex on the extracellular matrix should be considered with intact cells (myotubes, myofibers) embedded in a collagen hydrogel, and by applying traction force microscopy to quantify the traction forces in resting and field-stimulated hydrogels. The central question to be answered through these experiments

is whether the filamin C-integrin or the filamin C-Z-disk anchorage is more crucial in determining the compromised biomechanical properties, for instance, in p.W2710X-filamin C myopathy. In addition, after answering this fundamental biological question, the efficacy of therapeutic approaches to filamin C treatment in the murine models, that is, mild exercise regimes and the use of chemical chaperones, should be addressed. Of relevance and importance is linking these methods to imaging projects so as to define whether and under which manipulations filamin C also acts as a mobile fraction in the mutated phenotype, that is, translocating from the Z-disk to the I-band region, and how this affects biomechanical properties of muscle (Leber et al., 2016).

More recently, Kathage et al. (2017) showed that the filamin C-associated protein, BAG3 regulates protein synthesis through mechanical strain, and Collier et al. (2019) reported that phosphorylation of another filamin C-associated protein (HspB1) is responsible for mechanosensitive chaperone interaction with filamin C. In conclusion, more in-depth studies are needed to elucidate the effect of mechanical stress on the localization and dynamic behavior of other filamin C-associated proteins and their variants.

References

Almeida, F. V., G. Walko, J. R. McMillan, J. A. McGrath, G. Wiche, A. H. Barber and J. T. Connelly (2015). "The cytolinker plectin regulates nuclear mechanotransduction in keratinocytes." Journal of Cell Science **128**: 4475–4486.

Andrä, K., H. Lassmann, R. Bittner, S. Shorny, R. Fässler, F. Propst and G. Wiche (1997). "Targeted inactivation of plectin reveals essential function in maintaining the integrity of skin, muscle, and heart cytoarchitecture." Genes & Development **11**: 3143–3156.

Apte, K., R. Stick and M. Radmacher (2017). "Mechanics in human fibroblasts and progeria: Lamin A mutation E145K results in stiffening of nuclei." Journal Molecular Recognition **30**. doi: 10.1002/jmr.2580.

Bär, H., N. Mücke, P. Ringler, S. A. Müller, L. Kreplak, H. A. Katus, U. Aebi and H. Herrmann (2006). "Impact of disease mutations on the desmin filament assembly process." Journal of Molecular Biology **360**: 1031–1042.

Bär, H., M. Schöpferer, S. Sharma, B. Hochstein, N. Mücke, H. Herrmann and N. Willenbacher (2010). "Mutations in desmin's carboxy-terminal "tail" domain severely modify filament and network mechanics." Journal of Molecular Biology **397**: 1188–1198.

Bonakdar, N., J. Luczak, L. A. Lautscham, M. Czonstke, T. M. Koch, A. Mainka, T. Jungbauer, W. H. Goldmann, R. Schröder and B. Fabry (2012). "Biomechanical characterization of a desminopathy in primary human myoblasts." Biochemical and Biophysical Research Communications **419**: 703–707.

Bonakdar, N., A. Schilling, M. Spörrer, P. Lennert, A. Mainka, L. Winter, G. Walko, G. Wiche, B. Fabry and W. H. Goldmann (2015). "Determining the mechanical properties of plectin in mouse myoblasts and keratinocytes." Experimental Cell Research **331**: 331–337.

Bonne, G., M. R. Di Barletta, S. Varnous, H. M. Be`cane, E. H. Hammouda, L. Merlini, F. Muntoni, C. R. Greenberg, F. Gary, J. A. Urtizberea, D. Duboc, M. Fardeau, D. Toniolo and K. Schwartz

(1999). “Mutations in the gene encoding lamin A/C cause autosomal dominant Emery-Dreifuss muscular dystrophy.” Nature Genetics **21**: 285–288.

Brennich, M. E., U. Vainio, T. Wedig, S. Bauch, H. Herrmann and S. Köster (2019). “Mutation-induced alterations of intra-filament subunit organization in vimentin filaments revealed by SAXS.” Soft Matter **15**: 1999–2008.

Broers, J. L. V., E. A. G. Peeters, H. J. H. Kuijpers, J. Endert, C. V. C. Bouten, C. W. J. Oomens, F. P. Baaijens and F. C. S. Ramaekers (2004). “Decreased mechanical stiffness in LMNA-/- cells is caused by defective nucleo-cytoskeletal integrity: Implications for the development of laminopathies.” Human Molecular Genetics **13**: 2567–2580.

Brull, A., B. Morales-Rodriguez, G. Bonne, A. Muchir and A. T. Bertrand (2018). “The pathogenesis and therapies of striated muscle laminopathies.” Frontiers in Physiology **9**: 1533.

Buttgereit, A., C. Weber, C. S. Garbe and O. Friedrich (2013). “From chaos to split-ups-SHG microscopy reveals a specific remodelling mechanism in ageing dystrophic muscle.” The Journal of Pathology **229**: 477–485.

Buttgereit, A., C. Weber and O. Friedrich (2014). “A novel quantitative morphometry approach to assess regeneration in dystrophic skeletal muscle.” Neuromuscular Disorders **24**: 596–603.

Capetanaki, Y., R. J. Bloch, A. Kouloumenta, M. Mavroidis and S. Psarras (2007). “Muscle intermediate filaments and their links to membranes and membranous organelles.” Experimental Cell Research **313**: 2063–2076.

Charrier, E. E. and P. A. Janmey (2016). “Mechanical properties of intermediate filament proteins.” Methods in Enzymology **568**: 35–57.

Chatzifrangkeskou, M., D. Yadin, T. Marais, S. Chardonnet, M. Cohen-Tannoudji, N. Mougenot, A. Schmitt, S. Crasto, E. Di Pasquale, C. Macquart, Y. Tanguy, I. Jebeniani, M. Pucéat, B. Morales-Rodriguez, W. H. Goldmann, M. Dal Ferro, M. G. Biferi, P. Knaus, G. Bonne, H. J. Worman and A. Muchir (2018). “Cofilin-1 phosphorylation catalyzed by ERK1/2 alters cardiac actin dynamics in dilated cardiomyopathy caused by lamin A/C gene mutation.” Human Molecular Genetics **27**: 3060–3078.

Chatzifrangkeskou, M., D. Kah, J. R. Lange, W. H. Goldmann and A. Muchir (2020). “Mutated lamin A modulates stiffness in muscle cells.” Biochemical and Biophysical Research Communications **529**: 861–867.

Chevessier, F., J. Schuld, Z. Orfanos, A. C. Plank, L. Wolf, A. Märkens, A. Unger, U. Schlötzer-Schrehardt, R. A. Kley, S. von Hörsten, K. Markus, W. A. Linke, M. Vorgerd, P. F. van der Ven, D. O. Fürst and R. Schröder (2015). “Myofibrillar instability exacerbated by acute exercise in filaminopathy.” Human Molecular Genetics **24**: 7207–7220.

Clemen, C. S., D. Fischer, J. Reimann, L. Eichinger, C. R. Müller, H. D. Müller, H. H. Goebel and R. Schröder (2009). “How much mutant protein is needed to cause a protein aggregate myopathy in vivo? Lessons from an exceptional desminopathy.” Human Mutation **30**: E490–E499.

Clemen, C. S., H. Herrmann, S. V. Strelkov and R. Schröder (2013). “Desminopathies: Pathology and mechanisms.” Acta Neuropathologica **125**: 47–75.

Clemen, C. S., F. Stockigt, K. H. Strucksberg, F. Chevessier, L. Winter, J. Schütz, R. Bauer, J. M. Thorweihe, D. Wenzel, U. Schlötzer-Schrehardt, V. Rasche, P. Krsmanovic, H. A. Katus, W. Rottbauer, S. Just, O. J. Müller, O. Friedrich, R. Meyer, H. Herrmann, J. W. Schrickel and R. Schröder (2015). “The toxic effect of R350P mutant desmin in striated muscle of man and mouse.” Acta Neuropathologica **129**: 297–315.

Collier, M. P., T. R. Alderson, C. P. de Villiers, D. Nicholls, H. Y. Gastall, T. M. Allison, M. T. Degiacomi, H. Jiang, G. Mlynek, D. O. Fürst, P. F. M. van der Ven, K. Djinovic-Carugo, A. J. Baldwin, H. Watkins, K. Gehmlich and J. L. P. Benesch (2019). “HspB1

phosphorylation regulates its intramolecular dynamics and mechanosensitive molecular chaperone interaction with filamin C." Science Advances **22**: 5. eaav8421.
Cooper, G. M. (2000). Intermediate filaments. The cell, a molecular approach." Chapter 11/IV. Sunderland. Boston (MA), Sinauer Associates, Inc. (Oxford University Press). ISBN-10: 0-87893-106-6.
Dahl, K. N. and A. Kalinowski (2011). "Nucleoskeleton mechanics at a glance." Journal of Cell Science **124**: 675–678.
Diermeier, S., J. Iberl, K. Vetter, M. Haug, C. Pollmann, B. Reischl, A. Buttgereit, S. Schürmann, M. Spörrer, W. H. Goldmann, B. Fabry, F. Elhamine, R. Stehle, G. Pfitzer, L. Winter, C. S. Clemen, H. Herrmann, R. Schröder and O. Friedrich (2017a). "Early signs of architectural and biomechanical failure in isolated myofibers and immortalized myoblasts from desmin-mutant knock-in mice." Scientific Report **7**: 1391.
Diermeier, S., A. Buttgereit, S. Schürmann, L. Winter, H. Xu, R. M. Murphy, C. S. Clemen, R. Schröder and O. Friedrich (2017b). "Preaged remodeling of myofibrillar cytoarchitecture in skeletal muscle expressing R349P mutant desmin." Neurobiology of Aging **58**: 77–87.
Etienne-Manneville, S. (2018). "Cytoplasmic intermediate filaments in cell biology." Annual Review of Cell and Developmental Biology **34**: 1–28.
Fatkin, D., C. Mac Rae, T. Sasaki, M. R. Wolff, M. Porcu, M. Frenneaux, J. Atherton, H. J. Vidaillet, S. Spudich, I. de Girolami, U. J. Seidman and C. E. Seidman (1999). "Missense mutations in the rod domain of the lamin A/C gene as causes of dilated cardiomyopathy and conduction-system disease D." New England Journal of Medicine **341**: 1715–1724.
Fletcher, D. A. and D. Mullins (2010). "Cell mechanics and the cytoskeleton." Nature **463**: 485–492.
Friedrich, O., M. Haug, B. Reischl, G. Prölß, L. Kiriaev, S. I. Head and M. B. Reid (2019). "Single muscle fibre biomechanics and biomechatronics – The challenges, the pitfall and the future." The International Journal of Biochemistry & Cell Biology **114**: 105563.
Fürst, D. O., L. G. Goldfarb, R. A. Kley, M. Vorgerd, M. Olive and P. F. van der Ven (2013). "Filamin C-related myopathies: Pathology and mechanisms." Acta Neuropathologica **125**: 33–46.
Goldfarb, L. G. and M. C. Dalakas (2009). "Tragedy in a heartbeat: Malfunctioning desmin causes skeletal and cardiac muscle disease." The Journal of Clinical Investigation **119**: 1806–1813.
Goldman, R. D., M. M. Cleland, S. N. P. Murthy, S. Mahammad and E. R. Kuczmarski (2011). "Inroads into the structure and function of intermediate filament networks." Journal of Structural Biology **177**: 14–23.
Gomez, J., R. Lorca, J. R. Reguero, C. Moris, M. Martin, S. Tranche, B. Alonso, S. Igleas, V. Alvarez, B. Diaz-Molina, P. Avanzas and E. Coto (2017). "Screening of the filamin C gene in a large cohort of hypertrophic cardiomyopathy patients." Circulation: Cardiovascular Genetics **10**: e001584.
Haug, M., C. Meyer, B. Reischl, G. Prölß, S. Nübler, S. Schürmann, D. Schneidereit, M. Heckel, T. Pöschel, S. J. Rupitisch and O. Friedrich (2019). "MyoRobot 2: Advanced biomechatronics platform for automated, environmentally-controlled muscle single fiber biomechanics assessment employing inbuilt real-time optical imaging." Biosensors & Bioelectronics **138**: 111284.
Herrmann, H., S. V. Strelkov, P. Burkhard and U. Aebi (2009). "Intermediate filaments: Primary determinants of cell architecture and plasticity." The Journal of Clinical Investigation **119**: 1772–1183.
Herrmann, H. and U. Aebi (2016). "Intermediate filaments: Structure and assembly." Cold Spring Harbor Perspectives in Biology **8**: pii: a018242.
Herrmann, H., E. Cabet, N. R. Chevalier, J. Moosmann, D. Schultheis, J. Haas, M. Schowalter, C. Berwanger, V. Weyerer, A. Agaimy, B. Meder, O. J. Müller, H. A. Katus, U. Schlötzer-Schrehardt, P. Vicart, A. Ferreiro, S. Dittrich, C. S. Clemen, A. Lilienbaum and R. Schröder

(2020). “Dual functional states of R406W-desmin assembly complexes cause cardiomyopathy with severe intercalated disc derangement in humans and knock-in mice.” Circulation **142**: 2155–2171.

Hijikata, T., T. Murakami, M. Imamura, N. Fujimaki and H. Ishikawa (1999). “Plectin is a linker of intermediate filaments to Z-discs in skeletal muscle fibers.” Journal of Cell Science **112**: 867–876.

Hnia, K., C. Ramspacher, J. Vermot and J. Laporte (2015). “Desmin in muscle and associated diseases: Beyond the structural function.” Cell and Tissue Research **360**: 591–608.

Kathage, B., S. Gehlert, A. Ulbricht, L. Lüdecke, V. E. Tapia, Z. Orfanos, D. Wenzel, W. Bloch, R. Volkmer, B. K. Fleischmann, D. O. Fürst and J. Höhfeld (2017). “The co-chaperone BAG3 coordinates protein synthesis and autophagy under mechanical strain through spatial regulation of mTORC1.” Biochimica Et Biophysica Acta – Molecular Cell Research **1864**: 62–75.

Köster, S., D. A. Weitz, R. D. Goldman, U. Aebi and H. Herrmann (2015). “Intermediate filament mechanics in vitro and in the cell: From coiled coils to filaments, fibers and networks.” Current Opinion in Cell Biology **32**: 82–91.

Kolb, T., J. Kraxner, K. Skodzek, M. Haug, D. Crawford, K. K. Maaß, K. E. Aifantis and G. Whyte (2017). “Optomechanical measurement of the role of lamins in whole cell deformability.” Journal of Biophotonics **10**: 1657–1664.

Konieczny, P., P. Fuchs, S. Reipert, K. S. Kunz, A. Zeöld, I. Fischer, D. Paulin, R. Schröder and G. Wiche (2008). “Myofiber integrity depends on desmin network targeting to Z-disks and costameres via distinct plectin isoforms.” The Journal of Cell Biology **81**: 667–681.

Lammerding, J., P. C. Schulze, T. Takahashi, S. Kozlov, T. Sullivan, R. D. Kamm, C. L. Stewart and R. T. Lee (2004). “Lamin A/C deficiency causes defective nuclear mechanics and mechano-transduction.” The Journal of Clinical Investigation **113**: 370–378.

Lammerding, J., L. G. Fong and R. T. Lee (2006). “Lamins A and C but not lamin beta1 regulate nuclear mechanics.” Journal of Biological Chemistry **281**: 25768–25780.

Lange, J. R., J. Steinwachs, T. Kolb, L. A. Lautscham, I. Harder, G. Whyte and B. Fabry (2015). “Micro-constriction arrays for high throughput quantitative measurements of cell mechanical properties.” Biophysical Journal **109**: 26–34.

Lange, J. R., C. Metzner, S. Richter, W. Schneider, M. Spermann, T. Kolb, G. Whyte and B. Fabry (2017). “Unbiased high-precision cell mechanical measurements with micro-constrictions.” Biophysical Journal **112**: 1472–1480.

Lanzicher, T., V. Martinelli, L. Puzzi, G. Del Favero, B. Codan, C. S. Long, L. Mestroni, M. R. G. Taylor and O. Sbaizero (2015). “The cardiomyopathy lamin A/C D192G mutation disrupts whole-cell biomechanics in cardiomyocytes as measured by atomic force microscopy loading-unloading curve analysis.” Scientific Report **5**: 13388.

Lautscham, L. A., C. Kämmerer, J. R. Lange, T. Kolb, C. Mark, A. Schilling, P. L. Strissel, R. Strick, C. Gluth, A. C. Rowat, C. Metzner and B. Fabry (2015). “Migration in confined 3D environments is determined by a combination of adhesiveness, nuclear volume, contractility, and cell stiffness.” Biophysical Journal **109**: 900–913.

Leber, Y., A. A. Ruparelia, G. Kirfel, P. F. van der Ven, B. Hoffmann, R. Merkel, R. J. Bryson-Richardson and D. O. Fürst (2016). “Filamin C is a highly dynamic protein associated with fast repair of myofibrillar microdamage.” Human Molecular Genetics **25**: 2776–2788.

Lee, J. S., C. M. Hale, P. Panorchan, S. B. Khatau, J. P. George, Y. Tseng, C. L. Stewart, D. Hodzic and D. Wirtz (2007). “Nuclear lamin A/C deficiency induces defects in cell mechanics, polarization, and migration.” Biophysical Journal **93**: 2542–2552.

Lloyd, D. J., R. C. Trembath and S. Shackleton (2002). “A novel interaction between lamin A and SREBP1: Implications for partial lipodystrophy and other laminopathies.” Human Molecular Genetics **11**: 769–777.

Mitchell, M. J., C. Denais, M. F. Chan, Z. Wang, J. Lammerding and M. R. King (2015). "Lamin/ A deficiency reduces circulating tumor cell resistance to fluid shear stress." American Journal of Physiology-Cell Physiology **309**: C736–746.

Moir, R. D., T. P. Spann, H. Herrmann and R. D. Goldman (2000). "Disruption of nuclear lamin organization blocks the elongation phase of DNA replication." The Journal of Cell Biology **149**: 1179–1191.

Mücke, N., S. Winheim, H. Merlitz, J. Buchholz, J. Langowski and H. Herrmann (2016). "In vitro assembly kinetics of cytoplasmic intermediate filaments: A correlative Monte Carlo simulation study." PloS One **11**: e0157451.

Mücke, N., L. Kämmerer, S. Winheim, R. Kirmse, J. Krieger, M. Mildenberger, J. Baßler, E. Hurt, W. H. Goldmann, U. Aebi, K. Toth, J. Langowski and H. Herrmann (2018). "Assembly kinetics of vimentin tetramers to unit-length filaments: A stopped flow study." Biophysical Journal **114**: 2408–2418.

Na, S., F. Chowdhury, B. Tay, M. Ouyang, M. Gregor, Y. Wang, G. Wiche and N. Wang (2009). "Plectin contributes to mechanical properties of living cells." American Journal of Physiology-Cell Physiology **296**: C868–C877.

Nikolova, V., C. Leimena, A. C. McMahon, L. C. Tan, S. Chandar, D. Jogia, S. H. Kesteven, J. Michalicek, R. Otway, F. Verheyen, S. Rainer, C. L. Stewart, D. Martin, M. P. Feneley and D. Fatkin (2004). "Defects in nuclear structure and function promote dilated cardiomyopathy in lamin A/C-deficient mice." The Journal of Clinical Investigation **113**: 357–369.

Osmanagic-Myers, S., S. Rus, M. Wolfram, D. Brunner, W. H. Goldmann, N. Bonakdar, I. Fischer, S. Reipert, A. Zuzuarregui, G. Walko and G. Wiche (2015a). "Plectin reinforces vascular integrity by mediating crosstalk between the vimentin and the actin networks." Journal of Cell Science **128**: 4138–4150.

Osmanagic-Myers, S., T. Dechat and R. Foisner (2015b). "Lamins at the crossroads of mechano-signaling." Genes & Development **29**: 225–237.

Pfeifer, C. R., M. Vashisth, Y. Xia and D. E. Discher (2019). "Nuclear failure, DNA damage, and cell cycle disruption after migration through small pores: A brief review." Essays in Biochemistry: EBC20190007.

Schröder, R. and B. Schoser (2009). "Myofibrillar myopathies: A clinical and myopathological guide." Brain Pathology (Zurich, Switzerland) **19**: 483–492.

Schröder, R., A. Vrabie and H. H. Goebel (2007). "Primary desminopathies." Journal of Cellular and Molecular Medicine **11**: 416–426.

Schwartz, C., M. Fischer, K. Mamchaoui, A. Bigot, T. Lok, C. Verdier, A. Duperray, R. Michel, I. Holt, T. Voit, S. Quijano-Roy, G. Bonne and C. Coirault (2017). "Lamins and nesprin-1 mediate inside-out mechanical coupling in muscle cell precursors through FHOD1." Scientific Report **7**: 1253. 10.1038/s41598-017-01324-z.

Schween, L., N. Mücke, S. Portet, W. H. Goldmann, H. Herrmann and B. Fabry (2022). "Dual-wavelength stopped-flow analysis of the lateral and longitudinal assembly kinetics of vimentin." Biophysical Journal 121: 1–12.

Schürmann, S., S. Wagner, S. Herlitze, C. Fischer, S. Gumbrecht, A. Wirth-Hücking, G. Prölß, L. A. Lautscham, B. Fabry, W. H. Goldmann, V. Nikolova-Krstevski, B. Martinac and O. Friedrich (2016). "The Iso-stretcher: An isotropic cell stretch device to study mechanical biosensor pathways in living cells." Biosensors & Bioelectronics **81**: 363–372.

Selcen, D., F. Muntoni, B. K. Burton, E. Pegoraro, C. Sewry, A. V. Bite and A. G. Engel (2009). "Mutation in BAG3 causes severe dominant childhood muscular dystrophy." Annals of Neurology **65**: 83–89.

Spörrer, M., D. Kah, R. C. Gerum, B. Reischl, D. Huraskin, C. A. Dessalles, W. Schneider, W. H. Goldmann, H. Herrmann, I. Thievessen, C. S. Clemen, O. Friedrich, S. Hashemolhosseini,

R. Schröder and B. Fabry, (2022). “The desmin mutation R349P increases contractility and fragility of stem cell-generated muscle micro-tissues.” Neuropathology and Applied Neurobiology **48**: e12784.

Vignier, N., M. Chatzifrangkeskou, B. Morales-Rodriguez, M. Mericskay, N. Mougenot, K. Wahbi, G. Bonne and A. Muchir (2018). “Rescue of biosynthesis of nicotinamide adenide adenine dinucleotide protects the heart in cardiomyopathy caused by lamin A/C gene mutation.” Human Molecular Genetics **27**: 3870–3880.

Vorgerd, M., P. F. van der Ven, V. Bruchertseifer, T. Löwe, R. A. Kley, R. Schröder, H. Lochmüller, M. Himmel, K. Köhler, D. O. Fürst and A. Hübner (2005). “A mutation in the dimerization domain of filamin C causes a novel type of autosomal dominant myofibrillar myopathy.” American Journal of Human Genetics **77**: 297–304.

Wiche, G., H. Herrmann, F. Leichtfried and R. Pytela (1982). “Plectin: A high molecular weight cytoskeletal polypeptide component that copurifies with intermediate filaments of the vimentin type.” Cold Spring Harbor Symposia Quantitative Biology **46**: 475–482.

Winter, L., I. Staszewska, E. Mihailovska, I. Fischer, W. H. Goldmann, R. Schröder and G. Wiche (2014). “Chemical chaperone ameliorates pathological protein aggregation in plectin-deficient muscle.” The Journal of Clinical Investigation **124**: 1144–1157.

Winter, L. and W. H. Goldmann (2015). “Biomechanical characterization of myofibrillar myopathies.” Cell Biology International **39**: 361–363.

Winter, L., A. Unger, C. Berwanger, M. Spörrer, M. Türk, F. Chevessier, K. H. Strucksberg, U. Schlötzer-Schrehardt, I. Wittig, W. H. Goldmann, K. Marcus, W. A. Linke, C. S. Clemen and R. Schröder (2019). “Imbalances in protein homeostasis caused by mutant desmin.” Neuropathology and Applied Neurobiology **45**: 476–494.