

MINI-REVIEW

Intermediate filaments and cellular mechanics

Wolfgang H. Goldmann, PhD*

Department of Physics, Biophysics Group, Friedrich-Alexander-University Erlangen-Nuremberg, D-91052 Erlangen, Germany

Abstract

Intermediate filaments (IFs) are one of the three types of cytoskeletal polymers that resist tensile and compressive forces in cells. They crosslink each other as well as with actin filaments and microtubules by proteins, which include desmin, filamin C, plectin, and lamin (A/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

Introduction

Intermediate filaments (IFs) are found in many cell types and are part of the cellular cytoskeleton. They extend throughout the cytoplasm connecting the nuclear and cell membrane with actin filaments and microtubules and are responsible for cell morphology and mechanics (Fletcher and Mullins, 2010). Whilst the extra-sarcomeric IFs constitute a filamentous network in cells that connects membrane-anchored structures with Z-discs, some sarcomeric IF proteins integrate the cytoskeleton with organelles such as mitochondria and nuclei. Different IF protein types have been described in various cell types, and staggered assembly into proto-filaments gives them high tensile strength, which enhances their resistance to compression, stretching, and bending forces (Herrmann et al., 2009; Goldman et al., 2011; Herrmann and Aebi, 2016).

In the following paragraphs, I will briefly describe four important proteins (desmin, filamin C, plectin, and lamin A/C) of the IF network regarding their biological and mechanical function in living cells. These IF proteins have been reported to be important contributors to cell contractility and pre-stress and serve as molecular “guy wires” that facilitate transfer of mechanical loads between the cell surface and the nucleus and stabilize microtubules and actin filaments (Winter and Goldmann, 2015). A complete list of all IF proteins and their characteristics in various cell lines has been described by Cooper (2000).

Desmin molecule

The IF protein desmin is the most-studied disease entity in human myofibrillar myopathies (MFMs). The influence of desmin mutations was described from transfection experiments and in vitro assembly studies, indicating that the majority of desmin rod mutants are either incapable of forming de novo desmin IF networks, or constitute abnormal IF structures, or induce the collapse of a pre-existing IF network that leads to desmin-positive protein aggregates. These results showed that mutated desmin compromises filament-formation competence (Schröder et al., 2007; Schröder and Schoser, 2009; Clemen et al., 2015). From a clinical perspective the questions of whether these desmin mutants have a deleterious effect on the filament system in vitro and why it takes so long until clinical symptoms of progressive muscular damage, for instance, become apparent in humans remained.

It is unlikely that the complex human pathology is solely related to direct effects of desmin mutants on the assembly of desmin IFs. As an alternative explanation, desmin mutants may interfere in interactions with binding partners, thereby influencing the structural and functional organization of the extra-sarcomeric cytoskeleton as well as intracellular signaling cascades. This view is supported by the observation that mutations in genes encoding cytoskeletal linker proteins, for instance, in muscle cells that include plectin and filamin C, lead to similar

*Corresponding author: email: wgoldmann@biomed.uni-erlangen.de

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Abbreviations: cardiomyopathies; EBS-MD, epidermolysis bullosa simplex associated with muscular dystrophy; IFs, intermediate filament proteins; MFM, myofibrillar myopathies

cytoplasmic protein aggregates. Other contributing factors relate to metabolic abnormalities. Several studies demonstrated that the desmin–plectin–filamin C system plays an essential role in the subcellular positioning of mitochondria. Evidence for structural and biochemical mitochondrial dysfunction was found in human skeletal muscle from patients with desminopathy, plectinopathy, and filaminopathy (Goldfarb et al., 2008; Fürst et al., 2013; Winter and Wiche, 2013).

Desmin mechanics

Desmin has a tripartite structure comprising a central α -helical coiled-coil rod domain flanked by non- α -helical head and tail domains. The central rod domain plays a critical role in the dimerization and assembly process of desmin in the formation of the extra-sarcomeric cytoskeleton in all mature muscle cells (Bär et al., 2005, 2007; Herrmann et al., 2009). This filamentous structure forms a three-dimensional scaffold around myofibrillar Z-discs, thereby interlinking neighboring myofibrils and connecting the myofibrillar apparatus to nuclei, mitochondria, intercalated discs, and the cell membrane (Figure 1).

For instance, desmin knockout mice are viable and fertile, but they develop progressive muscle weakness and dystrophic alterations in cardiac and skeletal muscle. Since severe structural changes were most prominent in striated muscles, it was concluded that the lack of desmin results in an increased susceptibility of muscle fibers to physical strain during muscle contraction (Milner et al., 1996; Clemen et al., 2015).

We performed biomechanical experiments on wildtype and mutant desmin R349P myoblasts from mice, using the cell stretcher technique to apply external forces on adherent cells, the magnetic tweezer to detect the cell stiffness, and traction microscopy to determine the contractile forces of these cells. Our unpublished results showed higher vulnerability and internal cell stiffness, but lower active contractile forces in desmin mutants compared to wildtype cells. Meanwhile, Even et al. (2017) examining the mechanics of wildtype and mutant desmin p.D399Y mouse myoblasts using atomic force microscopy reported similar changes of cell stiffness. These authors argue that desmin aggregates in mutant cells are the responsible trigger for pathological symptoms in myofibrillar myopathies.

Previously, we had carried out mechanical measurements on isolated, primary muscle cells from patients with heterozygous R350P desmin mutation. Mutant cells showed a substantially higher stiffness compared to control cells, which directly implied that cell deformation leads to increased mechanical stress within the cytoskeleton and at the focal adhesion sites to a higher mechanical vulnerability (Bonakdar et al., 2012). These cells responded to cyclic mechanical stretch in time-matched control experiments (no stretch) with 2% dead cells in both control and diseased cells; however, the percentage of dead and detached cells after 1 h of cyclic 30% stretch was 11% in control cells and 17% in mutant desmin cells (Bonakdar et al., 2012). The results are in agreement with the hypothesis that mechanical stress increases in direct proportion to stiffness and stretch amplitude and that the higher cell death after stretch in mutant desmin cells must be due to higher stretch-induced

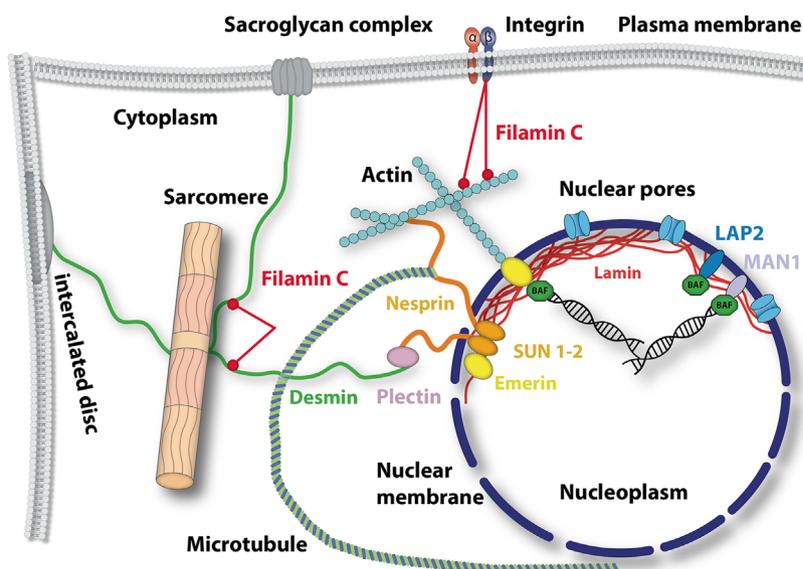


Figure 1 Schematic representation of IF proteins interacting in the cell. Taken and modified with permission from *Biology of the Cell*.

cell stress arising possibly from higher acto-myosin cytoskeletal pre-stress.

In striated muscle, desmin IFs interlink the contractile myofibrillar apparatus with mitochondria, nuclei, and the sarcolemma. The desmin network's pivotal role in myocytes is evident since mutations in the human desmin gene can cause severe myopathies and cardiomyopathies. We investigated skeletal muscle pathology in myofibers and myofibrils isolated from young hetero- and homozygous R349P desmin knock-in mice, which carry the orthologue of the most frequent human desmin missense mutation, R350P. We could demonstrate that mutant desmin alters myofibrillar cyto-architecture, markedly disrupts the lateral sarcomere lattice, and distorts myofibrillar angular axial orientation. Biomechanical assessment of small fiber and myofibrillar bundles showed alterations in mechanical stiffness, caffeine-induced force transients, and myofibrillar Ca^{2+} -sensitivity. These data suggested that mutated desmin markedly impedes myocyte structure and function at the pre-symptomatic stage of MFMs (Diermeier et al., 2017). Previous observations by Charrier et al. (2016) also showed that the expression of the E413K mutant desmin alters the traction forces generation of single myoblasts lacking organized sarcomeres.

Plectin molecule

Plectin is an important cytolinker of the IF protein desmin, which was first described and extensively characterized by Dr. Gerhard Wiche. Skeletal muscle studies have suggested that at least four plectin isoforms are responsible for differentially targeting and linking desmin IF networks to Z-disks, costameres, mitochondria, and the nuclear/ER membrane system, and plectin deficient cells show desmin aggregation and mitochondrial dysfunction. Plectin conditional and isoform-specific knockout mouse models were used to study the role of distinct isoforms in maintaining muscle fiber integrity. Together with data indicating similar disorders in Epidermolysis bullosa simplex associated with muscular dystrophy (EBS-MD) patients, these data established plectin as the major organizer of desmin IFs in myofibers (Wiche 1998; Winter and Wiche, 2013; Wiche et al., 2015).

Plectin mechanics

Plectin is the prototype of IF-based cytolinker proteins. It strengthens cells mechanically by interlinking and anchoring cytoskeletal filaments, and as a scaffolding and docking platform for signaling proteins, it controls cytoskeletal dynamics. We recently showed that pathogenic plectin mutations caused increased cell stiffness due to higher baseline contractile activation, which leads to higher

intracellular stress during cyclic stretch and consequently to higher stress vulnerability in muscle (Bonakdar et al., 2012, 2015; Winter et al., 2014; Osmanagic-Myers et al., 2015b). In a related set of experiments, we investigated the effect of a plectin knockout in mouse myoblasts. These experiments are particularly relevant because the same procedure for obtaining immortalized myoblasts with knockout mutations of extra-sarcomeric cytoskeletal proteins was followed in all subsequent studies.

Cell stiffness was approximately twofold decreased in the plectin knockout cells. In agreement with lower stiffness, plectin knockout 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 knockout cells were approximately 2.5-fold less contractile, which indicates a diminished cytoskeletal pre-stress that is probably the primary cause for lower stiffness of these cells (Bonakdar et al., 2015). In strong support of our hypothesis that cell death after stretching is caused by stretch-induced mechanical stress and, therefore, correlates with cell stiffness, we confirmed that the softer plectin knockout cells were approximately twofold 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.

Filamin C molecule

Filamin C is an actin-binding and regulatory protein that is closely associated with all stages of myofibril formation. The first mutation in the filamin C gene that caused 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 of the pathogenesis of filamin C myopathy was carried out by the group of Dr. Dieter 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, using the SIFT and other screening algorithms (Gomez et al., 2017).

Filamin C mechanics

In our research effort, we also addressed the fundamental question regarding the effects of filamin C mutations on the biomechanical function of skeletal muscle cells. We studied the molecular processes that contribute to the reduced mechanical stress resistance of diseased muscle cells. Biomechanical analyses were performed in combination with live-cell confocal microscopy and protein expression

studies on myoblasts derived from W2710X filamin C knock-in mice. First observations pointed in the direction that (i) filamin C mutant cells detach to 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. These observations were partly supported by findings of Chevessier et al. (2015) that mutant filamin C in muscle interferes with the mechanical stability and strain resistance of myofibrillar Z-discs.

Lamin (A/C) molecule

The protein lamin (A/C) connects via SUN1/2-nesprins to the nuclear membrane with IFs, the actin network, and microtubules of cells and determines their nuclear shape and mechanics (Lammerding et al., 2006; Lombardi et al., 2011). Lamin (A/C) deficiency leads to defects in nuclear structure and mechanics (i.e., increased nuclear deformation), decreased cytoskeletal stiffness, and dilative cardiomyopathy (Lammerding et al., 2004; Nikolova et al., 2004). Osmanagic-Myers et al. (2015a) also reported that in lamin A/C-deficient cells the nuclear mechanics is impaired. However, effects of lamin A/C overexpression are less well known. In melanoma cells overexpressing recombinant lamin A/C, reduced nuclear deformability was observed (Ribeiro et al., 2014).

Lamin (A/C) mechanics

Local force generation, dynamic modification of stiffness, and viscosity of cells and their responses to traction or compression forces are general hallmarks of cellular and tissue mechanics. These parameters have been examined by Lee et al. (2007) in lamin A-deficient mouse embryonic fibroblasts (MEFs). They reported that either the disassembly of actin filaments or microtubule networks led to the decrease of 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 non-association of the cytoskeleton with lamins was the trigger for cellular morphological and adhesive changes that could lead to reported fatal cardiomyopathies.

More 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 at 15% stretch, the

increased stiffness of the mutant HT1080 cells resulted in complete detachment of cells from the extracellular matrix, which confirmed 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 bring about cellular fragility and decrease the mechanical resistance to stress, which 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 responsible. Administering drugs, which inhibits 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, inactivating the ROCK-dependent regulator, formin responsible for remodeling actin rescued 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 (fibrosarcoma cell line HT-1080, the breast cancer cell line MDAMB-231, the lung carcinoma cell line A125, and primary mesenchymal cells from a patient with inflammatory duct [IFDUC1] breast carcinoma) that overexpressed lamin A migrating through 3D devices consisting of a linear channel length of 630 and 3.7 micrometer height with decreasing channel width from 11.2 to 1.7 micrometer (Lautscham et al., 2015). All cell lines showed reduced cell migration, which was attributed to higher cell stiffness and lower adhesiveness. To separate the influence of cell stiffness from other invasion-modulating cell properties, they increased the expression levels of lamin A, 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 influence of lamin A by the method of micro-constriction was investigated. To test the influence of lamin A overexpression on the overall cell mechanical properties, the stiffness and fluidity of various cells (leukemia cells, K562 and breast cancer cell line, MDA MB-231) was measured. Compared to wildtype cells, the stiffness of lamin A overexpressing cells increased significantly (Lange et al., 2015, 2017). Their data confirmed that lamin A contributes greatly to cell stiffness, but the method did not discriminate between the stiffness of the cell nucleus and the cytoskeleton.

They could not exclude the possibility that lamin A overexpression leads to an altered cytoskeletal structure and mechanics.

Conclusions

Recently identified disease mutations, for instance, in the IF protein desmin lead to skeletal and cardiac myopathies that correlate with pathological protein aggregation. Several researchers have elucidated the pathway (i.e., kinetics, dynamics, etc.) of protein assembly and found several indications that protein assembly pathways differ significantly from each other and that chaperones influence protein assembly.

The impact of mutations in muscle-specific IF proteins, which are mainly causative for the development of human muscular dystrophy, has been investigated extensively using recombinant human proteins. A major outcome of these studies was that mutations by no means necessarily lead to an impairment of IF assembly and that furthermore the severity of assembly disturbance cannot be predicted from simple rules. Several methods, including atomic force and electron microscopy as well as kinetic and mathematical analyses, were used.

We used a broad biophysical spectrum of techniques: (i) traction force microscopy to measure cellular adhesive and contractile forces; (ii) magnetic tweezer and nano-scale particle tracking to determine the micromechanics of single cells and cytoskeleton; and (iii) cell stretcher to characterize the response of cells to controlled mechanical loads (Bonakdar et al., 2015). We also analyzed the mechanical stiffness of isolated primary muscle cells from patients with myopathy, which showed a higher stiffness compared to control cells. In addition, we demonstrated that cell deformations lead to increased mechanical stress within the cytoskeleton and at the focal adhesion sites to a higher mechanical vulnerability.

To investigate the biomechanical properties of single myoblasts from MFM patients and animal models, cells were characterized by traction force microscopy to measure contractility, by magnetic tweezer micro-rheology to measure cellular biomechanics, and by cyclic stretch to measure their vulnerability. Mechanical vulnerability was estimated from cell viability measurements after uniaxial cell stretch experiments. The mechanism of mechanical stress vulnerability was investigated by confocal live-cell imaging to monitor the dynamic reorganization of force transmitting structures in focal adhesion contacts and in the cytoskeleton during external mechanical stimuli. The rationale for doing these experiments was that MFM mutations result in pathogenic protein aggregates and alterations of the plectin containing cytoskeleton even at early stages of muscle differentiation before the contractile apparatus is fully

developed. Myoblasts offer the advantage of representing a precursor cell system of muscle where cytoskeleton-membrane adhesions can be studied in isolation without interfering effects arising from contractile filaments in the adult cell. Pathogenic plectin mutations led to increased cell stiffness due to higher baseline contractile activation, higher intracellular stress during cyclic stretch, and therefore, higher stress vulnerability in muscle.

MFMs are generally associated with mutations in genes encoding cytoskeletal linker proteins. Most of these proteins connect adjacent myofibrils as well as myofibrils to Z-lines or other important cytoskeletal components and thus ensure proper anchorage in biomechanically active muscle. Disruptions of these linkages result in the disturbance of biomechanical properties that include elasticity and active force production. Although the common symptom in all patients with MFM is muscle weakness, there is almost no information at hand as to how muscle is affected at different structural and functional levels within the organ and as to the molecular cause of the muscle weakness.

In summary, as demonstrated here by many experiments, the lack or the mutation of IF proteins in muscle cells can impair their resistance to adhesive, stretching, elastic, and contractile forces, which leads in many cases to the deposition of protein aggregates. To what extent these aggregates influence the mechanics of cells is not yet known and still the focus of much research.

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