Chapter 12
Desmin Filaments and Desmin-Related Myopathy

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Introduction

At the histological level, muscle tissue is one of the four basic tissue types in the body, with the other three being epithelial, connective, and nervous tissues. Muscle tissue consists primarily of myocytes (muscle cells). Based on the morphology and functionality, muscle is further classified into three types: skeletal muscle, cardiac muscle, and smooth muscle. Skeletal and cardiac muscles together are also known as striated muscle as their myocytes or muscle fibers display microscopic striations. Among all cell types, myocytes especially striated myocytes have the highest content of cytoskeleton. This is because the primary function of myocytes is to generate mechanical force through contraction. The contractile apparatus (myofibril) in myocytes is primarily formed by the thick filament and the thin filament which are considered cytoskeleton. In addition to myofibrillar cytoskeleton, myocytes contain extra-myofibrillar cytoskeleton, including microtubules, microfilaments, and intermediate filaments (IFs), similar to non-muscle cells. In general IFs are formed by tissue-specific IF proteins. The most prominent IF protein expressed in muscle tissue is desmin.

Desmin was initially purified in 1976 from smooth muscle (chicken gizzard) [1]. As a cytoskeletal protein, desmin is a highly insoluble. Most buffers that solubilize myosin and the majority of actin are unable to solubilize desmin in muscle tissue; but desmin becomes soluble in presence of urea. Even in presence of 8M urea under a variety of conditions, desmin co-migrates with actin during purification. The employment of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) helped separate desmin from actin, leading to biochemical purification of desmin proteins. The first antibodies against desmin were then produced using

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the purified desmin protein, which allowed the spatial arrangement and structure of desmin filaments in striated and smooth muscles to be initially characterized via immunofluorescence staining. In striated muscle, desmin filaments were localized in between myofibrils at the z line level where they appear to link adjacent myofibrils to each other and to the sarcolemma. In cardiac muscle, desmin protein was also found to be enriched in the desmosome-like structure at the intercalated disc which couples adjacent cardiomyocytes head-to-head [1]. Lazarides and Hubbard termed the protein desmin (from the Greek, δεσμός = link, bond) to indicate the linking function that this molecule might have in muscle cells based on its immunofluorescence localization [1]. They named this protein as desmin for also denoting that this protein, besides being the subunit of the 10 nm filaments, is also a component of muscle desmosome-like structure in muscle cells [1].

In the past four decades, significant progresses are made in understanding of the structure and function of desmin protein and desmin IFs. Mouse genetic modification studies reveal desmin is not required for embryonic myogenesis but is essential to postnatal maintenance and function of striated muscles. The recent renaissance of research into desmin IFs was triggered by the first identification of mutations in the desmin gene (*DES*) and the genes encoding the partners of desmin IFs, such as αB-crystallin (*CRYAB*), in human familial desmin-related myopathy (DRM) in 1998 [2–4]. DRM is heterogeneous group of myopathies characterized pathologically by the presence of desmin-containing aberrant protein aggregates in myocytes. Since aberrant protein aggregation is a common process in all proteinopathies, insight gained from studying DRM pathogenesis serves also to improve our understanding of protein quality control and proteinopathy in general.

The Desmin Gene

Approximately 5 years after Capetanaki et al. had first identified the *DES* in chicken [5], Paulin and colleague cloned and characterized the human *DES* in 1989 [6]. In the human genome, there is a single copy of *DES*, which is 8.4 kbp in length, consists of nine exons and eight introns, and is localized to chromosome 2q35 [6, 7]. Northern blot analysis revealed that a single desmin mRNA of 2.2 kb is expressed in human striated and smooth muscles, which translates a protein of 470 amino acids (~53 kDa) [1, 6, 8]. From zebrafish to humans, DES is highly conserved.

A 280-bp muscle-specific enhancer located between −693 and −973 bp upstream of the transcription initiation site of human *DES* has been shown to confer high level expression of *DES* in both myoblasts and myotubes [9]. Further analysis revealed that this enhancer contains two different regions, one active in myoblasts and the other in myotubes. The myotube-specific region contains two E-box elements, providing the binding sites for MyoD1 and muscle-specific enhancer factor-2 (MEF-2), respectively; both are required for full enhancer activity. The myoblast-specific region is downstream of the myotube enhancer region, containing a region with homology to the M-CAT motif (at −587) and multiple regions harboring a GC-rich sequence sharing homology with the Krox binding site [10]. This combination of

promoter regulation may help explain why DES, but not contractile proteins, is expressed in myoblasts. A transgenic mouse model harboring a LacZ gene under the control of a promoter containing these regulatory regions shows transgene expression exclusively in skeletal muscle but not cardiac and smooth muscles [11], indicating that these regulatory elements are only sufficient to drive *DES* expression in skeletal muscle and that other regulatory regions must exist for cardiac and smooth muscle expression.

In mice, the first 85-bp upstream of the transcription initiation site of mouse *DES* contains an E box (E1), sufficient to drive low level of muscle-specific expression. An enhancer located between nucleotides −798 and −976 harbors another E box (E2) and a MEF-2 binding site, which drives high levels of *DES* expression. Both MyoD and myogenin are capable of binding to the E1 and E2; and MEF-2C, a myocyte-restricted member of the MEF-2 family can bind to the MEF-2 site in the enhancer [12]. Subsequent studies using mouse transgenesis demonstrated that a single MEF-2C binding site within 1kbp 5'-flanking sequence of mouse *DES* is sufficient to direct appropriate temporal expression of desmin in both cardiac and skeletal muscles during mouse embryogenesis [13].

In murine embryogenesis, desmin is detected as early as 8.25 days post coitum (d.p.c.) in the ectoderm, and then in the heart rudiment at 8.5 d.p.c., with elevated expression in cardiogenic cells thereafter [14]. Desmin protein begins to accumulate in somites at 9 d.p.c. and progresses in a rostro-caudal gradient with somatic maturation [14]. Desmin-positive myofibers are found in limb buds by 14 d.p.c. The levels of desmin expression in cardiac, skeletal, and smooth muscles stay high throughout subsequent embryonic development and into postnatal life [11]. Desmin is one of the earliest muscle-specific genes expressed during development; desmin protein has substantially accumulated in myogenic cells before myosin, actin, and titin begin to form the contractile apparatus [15–17].

Desmin Protein Structure and Filament Formation

Desmin belongs to the type III IF protein family. Other members of this family include vimentin, glial fibrillary acidic protein (GFAP), and peripherin. As illustrated in Fig. 12.1, a desmin protein molecule is comprised of three domains: an α-helical rod domain containing 304 amino acid residues, flanked by globular amino- and carboxyl-terminal structures which are known as the head and tail domains, respectively. The α-helical rod domain is interrupted by three short non-helical linker regions, resulting in four consecutive α-helical segments (1A, 1B, 2A, and 2B). The helical segments of the rod are highly conserved, sharing a sequence characteristic of a 7-residue (heptad) repeat pattern with a typical sequence of hydrophobic and hydrophilic amino acids. Each heptad forms a full helical turn and the heptad repeats of the α-helix in a desmin monomer guide two monomers into formation of a coiled-coil dimer, the basic unit of the filament. The equivalence of the eighth heptad of the 2B segment is interrupted by an insertion of four extra residues referred to as a 'stutter' (Fig. 12.1) [18]. The 'stutter' appears to be an obligatory

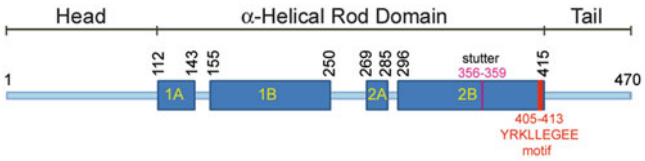

Fig. 12.1 A schematic illustration of desmin protein structure. Desmin protein consists of three domains: an α-helical rod domain containing 304 amino acid residues, flanked by globular amino- and carboxyl-terminal structures which are known as the head and tail domains, respectively. The α-helical rod domain is separated by three short non-helical linker regions, resulting in four consecutive α-helical segments (1A, 1B, 2A, and 2B). The heptad repeat pattern of the 2B helical segment is interrupted by an insertion of 4 amino acid residues at position 356–359, known as a stutter, and an YRKLLEGEE motif, marked by the *purple* and *orange boxes*, respectively in the illustration

feature of all IF proteins and its position is strictly conserved among different IF proteins. "Stutterless" desmin monomers created experimentally by insertion of the three "missing" amino acid to restore a continuous heptad repeat, lose the ability to anneal into longer filaments during filament elongation [19]. The presence of the stutter results in a slight unwinding of the coiled coil in the stutter vicinity. The local unwinding is apparently essential to the proper assembly of the filament. The YRKLLEGEE motif at the C-terminal end of the 2B segment is another well-studied structure. The coiled-coil interaction starts to loosen in this motif so that the two α-helices gradually separate and eventually bend away from each other at the EGEE level [20]. In vitro filament assembly studies reveal that the YRKLLEGEE motif directs the proper formation of tetramers and dictate the number of subunits per filament cross section. Approximately 30 % of the 'tail' domain is constituted by β-sheets, with the remainder of the domain bearing predominantly random structure and lacking the heptad repeat pattern. The tail domain participates in the longitudinal head-to-tail assembly of tetramers [21] and is involved in the control of lateral packing, as well as stabilization and elongation of the higher order filament structures [22, 23]. Another major function of the tail is to interact with other cytoskeletal proteins, which helps establish a cytoplasmic IF network [24].

The Desmin IF Network

In mature striated muscle, desmin IFs link myofibrils to each other at the z-disc level, to the sarcolemma, and to the nuclear envelope (Fig. 12.2) [25]. Desmin filaments surround each myofibril at the z-disc level where they interact with α-actinin

Fig. 12.2 (continued) the nuclear pore. The longitudinal component of desmin filament networks lay in the inter-myofibrillar space where they interact with and, help the positioning of, mitochondria (**b**) A confocal micrograph of a mouse ventricular myocardial section immunofluorescence-stained for desmin (*green*). Desmin staining displays a striated pattern and is enriched at the intercalated discs

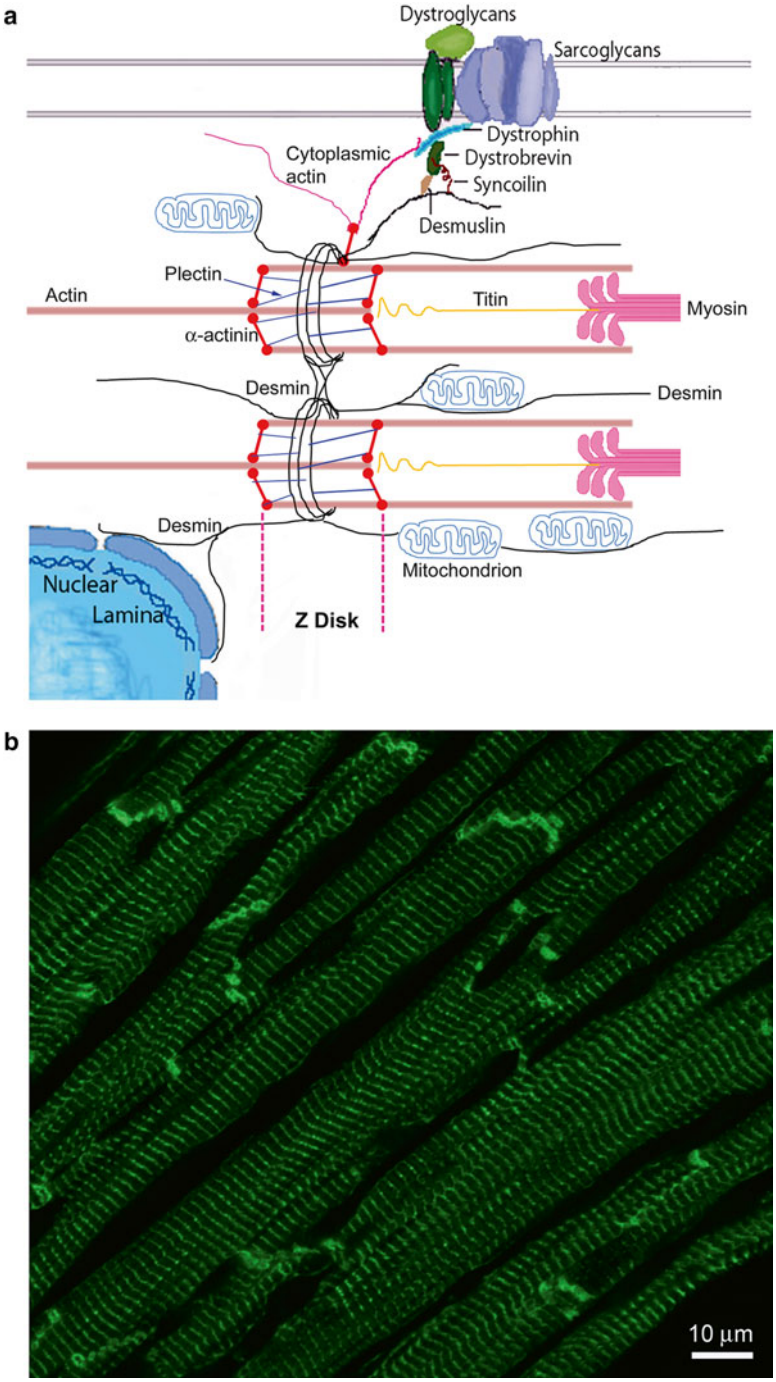

Fig. 12.2 Desmin filament distribution in striated muscle. (**a**) A schematic illustration of desmin filaments in relation to the z-disc of myofibrils, dystrophin complex, the nuclear envelop, and mitochondria. Desmin filaments surround each myofibril at the z-disc level and interact with α-actinin through plectin; desmin filaments interact with dytrobrevin in the dystrophin complex via syncoilin and desmuslin; and desmin filaments insert into the nuclear envelop at the proximity of

through plectin (isoforms 1d and 1f) [26]; desmin IFs interact with dytrobrevin in the dystrophin complex via syncoilin and desmuslin [27], thereby connecting to dystroglycans and sarcoglycans on the cell membrane; desmin IFs also associate with integrin via interaction with subsarcolemmal cytoskeleton and costameres; and lastly desmin filaments insert into the nuclear envelop at the proximity of the nuclear pore. Plectin (isoform 1b) also mediates the interaction of desmin filaments with mitochondria [26]. The longitudinal components of the desmin network insert into the desmosomes of the intercalated disc via desmoplakin. Desmin is highly enriched in desmosomes. Desmin showed a normal intracellular distribution but failed to localize at the intercalated discs in the myocardium from a patient with homozygous C-terminal truncation of desmoplakin [28]. Some data suggest that desmin filaments interact with the nuclear lamina through the nuclear pore [25, 29]. The postulated physical interaction is difficult to prove but a functional relationship is well implicated. For example, transgenic ablation of the gene encoding lamin A/C, the major proteins forming the nuclear lamina, produced a typical dilated cardiomyopathy (DCM) with markedly altered distribution of the desmin IF network and its relationship with the nuclear pores [30], suggesting an interaction between desmin IFs and lamin A/C and potential significance of this interaction in mechanotransduction. The unique and ubiquitous distribution of the desmin IF network has led to the hypothesis that the desmin IF network plays a role in the underlying structural integrity of a muscle cell, as well as participating in the signaling processes necessary for integration of cellular responses to external and internal stimuli [29]. However, this hypothesis has not been fully tested.

Like IFs in epithelia, desmin filaments in myocytes are mainly organized by plectin, a 500 kDa cytolinker protein. A study of conditional knockout of the plectin gene in striated muscles in mice demonstrates that plectin deficiency prevents desmin filaments from attaching to Z-disks, costameres, mitochondria, and the nuclear envelop, causing the formation of desmin aggregates of distinct morphology and in distinct cytoplasmic compartments, depending on which plectin isoforms are missing [26]. Striated muscle expresses two major plectin isoforms, plectin 1d and 1f, which specifically target and link desmin filaments to Z-disks and costameres, while plectin 1b forges a linkage to mitochondria [26]. On the other hand, desmin filaments also act as a scaffold for its interacting partner proteins; hence loss of desmin or altered distribution of desmin can cause changes in the expression and distribution of its partner proteins in the cell. For example, in desmin null mice, syncoilin was markedly decreased in skeletal muscle, disappeared from sarcomeric z-lines and neuromuscular junctions, and relocated from the sub-sarcolemmal cytoskeleton to the cytoplasm [31]; however, immunofluorescence microscopy revealed that knockout of syncoilin did not appear to discernibly change the distribution of desmin in striated muscle cells [32].

Desmin Loss of Function

As one of the earliest expressed genes during embryonic myogenesis, *DES* was shown by earlier studies using cell culture systems to be required for myocyte differentiation and myogenesis [33, 34]; however, this proposition is not proven by subsequent in vivo desmin loss of function studies. Two mouse models of germline knockout of *DES* were generated by two independent groups and both showed that desmin-null mice are viable and fertile [35, 36]. Studies of these desmin-null mice have yielded significant insight into the function of desmin IFs. Desmin is dispensable for the formation of skeletal, cardiac and smooth muscles during embryonic development but desmin null mice do display postnatal progressive structural disintegration and functional impairment in all three types of muscle [35, 36]. Adult desmin-null mice often show a slightly smaller body size, tend to be less strong and quicker to become fatigued, and show a significantly shortened lifespan (~12-months vs. 2 years in wild type), compared with their wild type littermate controls [35–39]. These general abnormalities observed in desmin-null mice are underlined by specific pathologies occurred in cardiac, skeletal, and smooth muscles, including life-threatening cardiomyopathy.

Consequence of Desmin Loss of Function in Cardiac Muscle

Desmin-null mice develop cardiomyopathy, the primary cause of their premature death. A prominent myocardial lesion in desmin-null hearts is focal cardiomyocyte degeneration, necrosis, macrophage infiltration, calcification, and fibrosis. Yellowish-white lesions ranging from single or multiple spots to confluent areas corresponding to calcification at the surface of the heart are macroscopically visible as early as 2 weeks postnatal. These lesions seem to affect more frequently the free wall of the right ventricle and the right ventricle side of the interventricular septum. At the microscopic level, cardiomyocyte degeneration can be detected as early as postnatal day 5 and throughout the heart [39]. Electron microscopy discerns alterations in the intercalated disc and sarcolemma of cardiomyocytes. Myofibril disorganization and abnormal nuclear and mitochondrial morphology and positioning are also detected via ultrastructural examination of the desmin-null hearts [40]. Cardiac injury caused by desmin deficiency triggers reactivation of the fetal gene program and the loss of cardiomyocytes renders the remaining cardiomyocytes to undergo hypertrophy and impairs cardiac mechanical function; ultimately, desmin-null mice develop DCM and congestive heart failure [38]. Cardiomyocyte-restricted overexpression of desmin completely rescued the cardiac pathology in desmin null mice [41], demonstrating that cardiac lesions observed in desmin-null mice are cardiomyocyte autonomous, not secondary to vascular abnormalities. Nevertheless, expression of

desmin is high in microarteries, where desmin plays a role in generating both passive and active tension [42].

According to some reports, the earliest ultrastructural defects of desmin-null cardiomyocytes are observed in mitochondria. In addition to mitochondrial clumping, extensive mitochondrial proliferation, swelling, and matrix degeneration are observed in a fraction of cardiomyocytes deficient of desmin, particularly after exercise. Functionally, no difference was discerned in the in vitro maximal rates of respiration in isolated cardiac mitochondria from desmin-null and wild-type mice; however, ADP-stimulated mitochondrial respiration in situ using saponin skinned muscle fibers was significantly reduced in cardiac and soles muscles from desmin-null mice, compared with wild type control [43]. Bcl-2 is an integral outer membrane protein of mitochondria that protects against apoptosis without affecting mitochondrial function. By cross-breeding desmin-null mice with Bcl2 overexpression transgenic mice, Weisleder et al. found that mitochondrial abnormalities in desmin-null hearts were remarkably ameliorated by Bcl-2 overexpression and, more intriguingly, that the correction of mitochondrial defects was associated with reduction in fibrotic lesions in the myocardium, prevention of cardiac hypertrophy, restoration of cardiomyocyte ultrastructure, and significant improvement of cardiac function. Furthermore, loss of desmin was found to diminish the capacity of mitochondria to resist exposure to calcium, a defect that was also partially restored by Bcl-2 overexpression [44]. These findings demonstrate that desmin filaments are essential for the positioning and functioning of mitochondria and mitochondrial dysfunction is a major cause of cardiomyopathy in desmin-null mice.

Consequence of Desmin Loss of Function in Skeletal Muscle

Skeletal muscle formation starts with the progenitor cell commitment to the myogenic lineage, myoblast proliferation, differentiation, and fusion to form first primary, and then secondary myotubes. As the primary myotube forms, new generations of myoblasts cluster around the primary myotube and use it as a scaffold to form secondary myotubes. Detailed analyses of myogenesis in mouse embryos have revealed that somites and myotomes form normally and mononucleate muscle precursor cells migrate normally in the absence of desmin. The assembly of sarcomeres and myofibrils does not seem to differ between desmin-null and wild type littermate embryos [37]. However, like cardiac muscle, alterations in skeletal muscle are discernible in desmin-null mice soon after birth. These pathological changes including focal areas of muscle degeneration, regeneration involving satellite cell activation and formation of new fibers, and fibrosis are most prominent in the highly solicited muscles, such as soleus (a weight-bearing muscle) or diaphragm and tongue (both very active muscles). Faulty myofibrillogenesis was frequently observed in regenerating myotubes and fibers from postnatal day 11 up to 12 weeks, suggesting that the desmin filaments are not required in embryonic muscle formation but are needed for a proper myofibrillar assembly during postnatal regeneration. This is perhaps because

additional mechanical stress is applied to these muscles after birth as they regenerate, which would not occur in utero. At 2 months of age, the force generated by the soleus of desmin-null mice is significantly less than in the control mice. At 5 months, the soleus of desmin-null mice is no longer able to respond to the stimulation and generate very little force. Defective mitochondrial activity may be an underlying cause; both NADH staining and ultrastructural examinations show large accumulations of mitochondria in muscle fibers deficient of desmin. Hence, it is generally concluded that desmin is not required for the proliferation and commitment of the early myoblasts to the myogenic lineage or for their migration, fusion, and subsequent organization of the muscle fiber before birth; however, after birth, it is not only essential to maintaining structural integrity in mature muscles but also important for muscle regeneration.

Consequence of Desmin Loss of Function in Smooth Muscle

Desmin protein was first isolated from smooth muscle [1]. It was determined later on that in smooth muscle cells, desmin is associated with the dense body, the smooth muscle analogue of the z-disk of striated muscle. Wede et al. analyzed the mechanical property of the aorta, the mesenteric artery, and resistance arteries in desmin-null mice [42]. For aorta and mesenteric artery, passive or active circumference- stress relations were not different between desmin-null and wild type mice. Both passive and active stresses in the microarteries were lower in the desmin-null group. Thus, desmin filaments do not seem to play a major role in the mechanical properties of the large elastic and muscular arteries where desmin expression is relatively low. In the microarteries, which contain a greater amount of desmin protein, desmin IFs show discernible contribution to both passive and active tension.

Asthma is one of the common chronic obstructive pulmonary diseases with airway inflammation and abnormal airway smooth muscle contraction, which is due to an intrinsic abnormality of the airway smooth muscle cells (ASMCs). ASMC hyperplasia and hypertrophy are key determinants of airway remodeling and hyperresponsiveness, characteristic of severe asthma and other chronic obstructive pulmonary diseases. Analysis of bronchial biopsies of asthmatic patients reveals a negative correlation between desmin expression in ASMCs and airway hyperresponsiveness [45], suggesting an important role for desmin in ASMC homeostasis. Indeed, experiments comparing desmin-null mice with wild type mice demonstrate that desmin is a load-bearing protein that increases the stiffness of the airways and the lung and modulates airway contractile response [46]. A subsequent study shows that desmin deficiency induces hypertrophy of ASMCs via up-regulation of microRNA-26a (miR-26a) which targets glycogen synthase kinase-3β, demonstrating a novel role for desmin as an anti-hypertrophic protein necessary for ASMC homeostasis and identifying desmin as a novel regulator of microRNA [47].

The role of desmin IFs in smooth muscle hypertrophy has also been examined in a urinary bladder growth model created by partial obstruction of the urethra.

The results indicate that desmin is not required for urinary bladder smooth muscle growth but plays a role in active force transmission and maintenance of wall structural integrity during growth of urinary bladder [48].

Taken together, the desmin loss of function studies have provided strong support for the hypothesis proposed originally by Lazarides [49] that desmin distributed in the intracellular space functions to link the Z disks together and to the membrane, and is important to maintain structural integrity of the muscle.

Desmin-Related Myopathy (DRM)

Desmin-related myopathy (DRM), also known as desmin myopathy, is a heterogeneous group of myopathies that have a shared pathological characteristic: the presence of desmin-positive aberrant protein aggregates in muscle cells. Biopsies of the affected skeletal and cardiac muscles display intrasarcoplasmic areas containing amorphous eosinophilic deposits which are immunostaining positive for desmin. Electron microscopic examination reveals that these abnormal structures at the ultrastructural level display as electron-dense granular or granulofilamentous material in the intermyofibrillar space; physical contact is sometimes observed between the granulofilamentous material and the z line of the sarcomere. Myofibril organization is apparently altered as evidenced by wavy z lines and z disc widening/streaming (Fig. 12.3) [50].

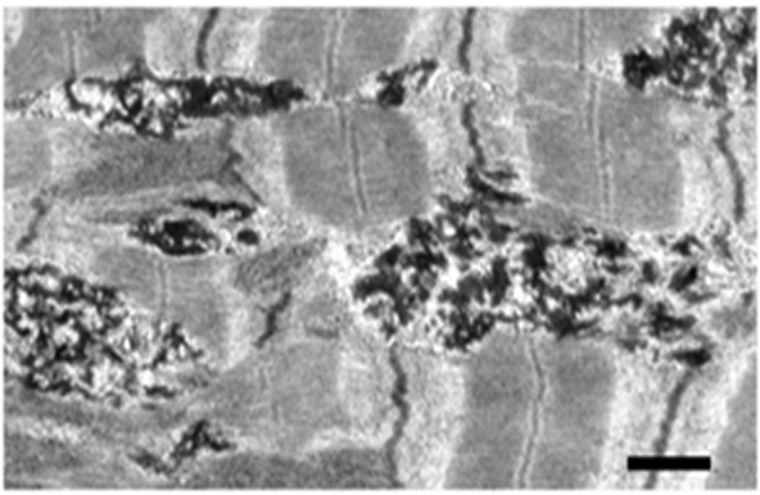

Fig. 12.3 An electron microphotograph of skeletal muscle biopsy from a human DRM patient heterozygous for a missense mutation (Q348P) in the desmin gene. Note that the myofibril organization shows dramatic changes and that Z line widening/streaming as well as accumulation of granular-filamentous materials are prominent. Scale bar = 1 μm. (Adopted from Fichna JP et al. PLoS One 2014; 9: e115470) [50]

DRM belongs to myofibrillar myopathy which is an even more heterogeneous group of muscle disorders featured by the presence of myofibrillar proteins positive inclusions and myofibrillar disintegration and disorganization. These inclusions are desmin-positive as well. Typically, DRM presents with muscle weakness initially in distal muscles, which slowly spreads to affect truncal, neck-flexor, facial, bulbar and respiratory muscles [51]. DRM can present as isolated skeletal myopathy or isolated cardiomyopathy but more often it presents in the form of combined skeletal and cardiac myopathy, with smooth muscle sometime being involved as well [52]. Cardiomyopathy in DRM is known as desmin-related cardiomyopathy (DRC). DRC can present phenotypes of hypertrophic, dilated, or restrictive cardiomyopathies. Conduction blocks and arrhythmias resulting in sudden death are observed as a major clinical manifestation of DRC. Age of disease onset varies from early childhood to mid-aged adult, seemingly depending on the type of inheritance, location of the causative mutation, and the gene mutated. Perhaps for the same reasons, the rate of disease progression is not quite uniformed.

Human Genetics of DRM

The pattern of inheritance pattern in familial DRM includes autosomal dominant or autosomal recessive. However, many DRM cases have no family history, at least some of which de novo *DES* mutations are identified. The first batch of *DES* mutations were reported in 1998 by Goldfarb et al. [2]; through genetic linkage analysis, they associated mutations in the highly conserved carboxyl-terminal end of the rod domain (2B segment) with two families with desmin-related cardiac and skeletal myopathy. They identified a heterozygous A337P mutation in a family with an adult-onset DRM and compound heterozygosity for two other mutations, A360P and N393I, in a second family with childhood-onset aggressive course of DRM. Approximately 1 month after Goldfarb's report, a putative 7-amino acid (R173-E179) deletion in the 1B segment of desmin rod domain was identified in a patient with generalized myopathy by MunÄoz-MaÂrmol et al. [3]. A wild type allele of *DES* was not found in this patient's genome; hence, this patient might be either hemizygous or homozygous for this mutation [3]. As summarized in Fig. 12.4, a large number of mutations have been identified in *DES*, including point substitutions, insertion, small in-frame deletions and a larger exon-skipping deletion. Most mutations are located in the highly conserved alpha-helical rod domain of desmin although mutations in the head and tail domains as well as the linker regions are also common.

Many of the missense mutations result in replacement of the original amino acid into proline, a known helix breaker. Since the helical structure in the rod domain is essential to the orderly polymerization of desmin protein molecules to form desmin filaments, mutations that destroy the α-helixes are believed to impair desmin filament formation. Indeed, studies of mutant desmin transfected cell cultures demonstrate that mutant desmin proteins are often incapable of assembling normal IF but

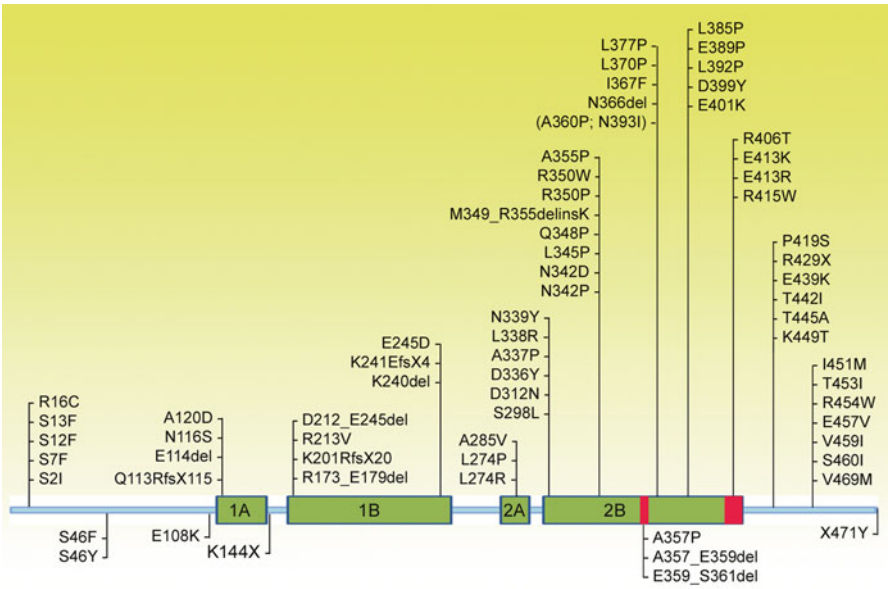

Fig. 12.4 A summary of DES mutations in relation to DES protein domain structure. A total of 67 mutations are identified so far with 8 in the head, 14 in the tail, and 45 in the rod domains

are usually able to disrupt a pre-existing filamentous network in a dominant-negative manner (see section "Disruption of the Desmin Filament Network in DRM").

Point substitutions can also result in premature stop codon and thereby truncation of the protein, in addition to missense mutant proteins. Insertions cause frameshift and truncation. All these situations are observed in DRM-associated *DES* mutations. In frame deletion can result from not only a small deletion in a coding exon but also splice mutations in introns of *DES* (Fig. 12.4). For example, splice site mutations in intron 2 or 3 that flank exon 3 result in deletion of 32 amino acids encoded by exon 3 [53]. Disease caused by mutations in *DES* is known as desminopathy to differentiate from the DRM caused by mutations in other genes.

In a large French pedigree with DRM, no mutation in *DES* was detected but Vicart et al. have identified an R120G missense mutation in the αB-crystallin gene (*CRYAB*) in this pedigree [4]. CRYAB was initially found in the lens of eyes over a century ago but studies published in 1980s have unraveled that CRYAB is also constitutively expressed in many non-lenticular tissues, especially in cardiac and skeletal muscles [54]. CRYAB is a highly conserved protein. Human CRYAB contains 175 amino acid residues with a molecular weight of 20 kDa. It turns out CRYAB is a member of the small heat shock protein family, is one of the most expressed cytosolic non-myofibrillar proteins in cardiomyocytes [54]. CRYAB had been shown to interact with desmin and actin in myocytes, especially under stress conditions. Muscle fibers from patients harboring $CRYAB^{R120G}$ were shown to contain aberrant protein aggregates that are immunopositive for both DES and CRYAB.

Table 12.1 Human disease-linked *CRYAB* mutations

Nucleotide change	Amino acid change	Type of inheritance	Muscle affected	References
c.358A > G	R120G	AD	HCM, SkM, PPC	[4]
c.325G > C	D109H	AD	HCM, SkM, PPC	[55]
c.460G > A	G154S	AD	DCM	[56]
c.470G > A	R157H	AD	DCM	[57]
c.451C > T	Q151X	AD	SkM	[58]
c.464delCT	L155fs_163X	AD	SkM	[58]
c.60delC	S21Afs_24X	AR	SkM	[59]
c.343delT	S115Pfs_129X	AR	SkM	[60]
c.450delA	K150Nfs_184X	AD	PPC	[61]
c.58C > T	P20S	AD	PPC	[62]
c.59C > G	P20R	AD	PPC	[63]
c.557G > A	A171T	AD	cataract	[64]

AD autosomal dominant, *AR* autosomal recessive, *SkM* skeletal myopathy, *HCM* hypertrophic cardiomyopathy, *DCM* dilated cardiomyopathy, *PPC* posterior polar cataract

Hence, CRYAB^{R120G} represents the first mutation in molecular chaperones linked to muscle disease. To date, several additional mutations in *CRYAB* have been linked to DRM. Since the identification of the R120G missense mutation, at least nine additional *CRYAB* mutations have been reported to associate with human disease (Table 12.1), including additional missense mutations as well as truncation and extension due to frame shift resulting from nucleotide deletion. Most of the mutations identified so far are autosomal dominant but autosomal recessive inheritance is also seen in two truncation mutations that linked to families with primarily skeletal myopathy. Patients with *CRYAB* mutations can develop isolated cardiomyopathy (primarily DCM), isolated skeletal myopathy (myofibrillar myopathy), isolated posterior polar cataract type 2 (PPC2), or all three in combination. Overall, all diseases caused by mutations in *CRYAB* is also known as αB-crystallinopathy.

In addition to *DES* and *CRYAB* mutations, mutations in a number of other genes that encode partner proteins of DES, such as myotilin, Z-band alternatively spliced PDZ-containing protein (ZASP), filamin C (FLNC), Bcl-2-associated athanogene-3 (BAG3) [65], are linked to myofibrillar myopathy to which DRM belongs.

Transgenic Mouse Models of DRM

The mechanisms by which DRM-linked genetic mutations cause pathology in DRM patients have been investigated primarily using mouse transgenics and cell cultures. The author of this Chapter and his colleague created the first mouse model of DRC by cardiomyocyte-restricted overexpression of a murine 7-amino-acid (R172-E178) deletion mutant desmin (known as D7-des) [66], which is the mouse homologue of human R173-E179 deletion mutant DES linked to DRM [3]. The D7-des transgenic

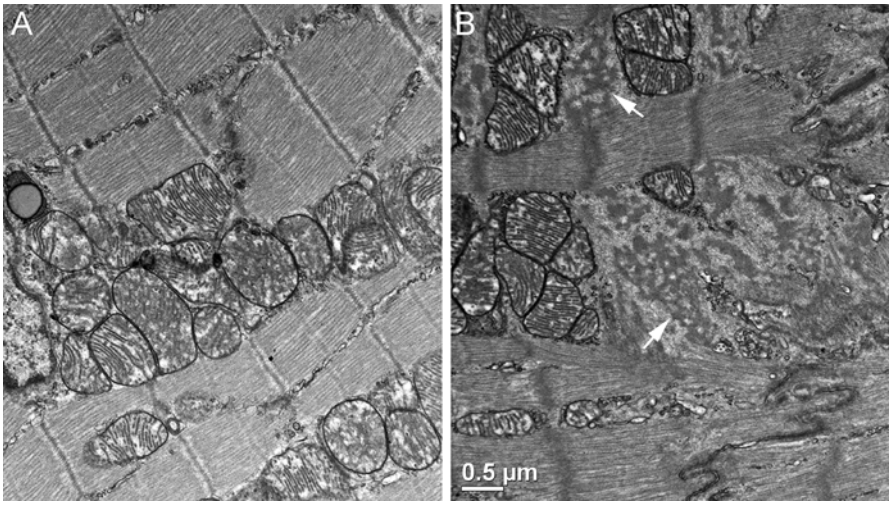

Fig. 12.5 Ultrastructural analysis of DRC mouse myocardium. Longitudinal sections of myocardial specimen from an adult non-transgenic mouse (**a**) and a littermate D7-des transgenic mouse (**b**) were analyzed. *Arrows point* to aberrant granular-filamentous desmin aggregates in the intermyofibril space

mice displays the characteristic intrasarcoplasmic electron-dense granulofilamentous materials in the intermyofibrillar space in the heart (Fig. 12.5) and marked cardiac hypertrophy and cardiac dysfunction, recapitulating main aspects of human DRC. Subsequently, several additional transgenic mouse models expressing other *DES* mutations have also been reported [67, 68]. Notably, an R349P desmin knock-in mouse model, which harbors the ortholog of the most frequently occurring human *DES* missense mutation R350P, has recently been generated [69], providing an animal model arguably most closely mimicking human DRM for investigating the pathogenesis of this mutant desmin in vivo.

Wang et al. also developed the first transgenic mouse model of CRYAB^{R120G}-based DRC via the mouse *myh6* promoter-driven cardiac-specific overexpression of murine cDNA encoding CRYAB^{R120G} [70]. In multiple stable lines of the CRYAB^{R120G} transgenic mice, aberrant protein aggregates immune-positive for DES and CRYAB are detected in cardiomyocytes throughout the heart. The rate of disease progression in this model depends on transgene expression level which is transgene copy number dependent in the *myh6* promoter-driven transgenics. In a stable line harboring three copies of the transgene, DRC progression can be divided into three distinct stages. At 1 month of age, these CRYAB^{R120G} mice show no apparent cardiac morphological and functional changes except for the presence of aberrant protein aggregates characteristic of DRM in cardiomyocytes; at 3 months, concentric cardiac hypertrophy is clearly discernible with compensated systolic function but impaired diastolic function; by 6 months, typical DCM and congestive heart failure are developed and the mice die prematurely around this age with an average lifespan of

~6 to 7 months. The disease progression in a stable transgenic line harboring one copy of the transgene is much slower [70]. Six years after the report of this first CRYAB^{R120G} mouse model, Rajasekaran et al. described another similarly developed CRYAB^{R120G} transgenic mouse model in which the cDNA of human *CRYAB* was used [71]. In fact, the amino acid sequence of CRYAB is highly conserved between mice and humans with both being 175 amino acid residues long with only 4 amino acids being different but all quite distal to the R120G mutation site. Not surprisingly, this transgenic mouse expressing human CRYAB^{R120G} displays exactly the same pathology as the strain expressing murine CRYAB^{R120G}. More recently, a mouse strain with the R120G mutation knocked in the exact locus of the mouse *cryab* gene, which should mimic most closely the human genetic alteration, has been reported. Initial characterization of this knock-in mouse shows that CRYAB^{R120G} is capable of causing skeletal myopathy and cataract in a dominant manner [72].

Disruption of the Desmin IF Network in DRM

As described in earlier sections, desmin-null mice develop generalized myopathy affecting skeletal, cardiac and smooth muscle structure and functions. Some of the desmin loss-of-function phenotypes resemble the manifestations of DRM [69]. This suggests that loss or disruption of the normal desmin IF network is likely an important pathogenic mechanism of DRM-linked *DES* mutations.

Based on a "time-lapse" electron microscopy of the in vitro assembly of desmin filaments from denatured purified desmin proteins, the assembly process can be divided into four stages: tetramer formation, unit-length filament (ULF) formation, longitudinal annealing and radial compaction, and IF network formation [73]. A seminal study by Bar et al. has analyzed 14 DES missense mutations and showed that two of them (A213V, E245D), both residing in the 1B section of the rod domain, can assemble into morphologically normal IFs that are indistinguishable from IFs formed by wild type DES, whereas four mutations residing in the 2B section (A360P, Q389P, N393I, and D399Y) can also form seemingly normal IFs but these IFs display subtle and yet discernible alterations in morphology and physical property [73]. Moreover, the remaining eight mutants interfere with the assembly process at distinct stages. The L385P and R406W mutants can yield apparently normal ULF but show defective longitudinal annealing and radial compaction; filament assemblies formed by A337P, N342D, or A357P -DES show enhanced stickiness and eventually lead to large aggregates; and for L345P, R350P, or L370P-DES, the assembly can be initiated and progresses to the ULF state but after stalling at ULF briefly, the ULF-like structure rapidly breaks down into small aggregates [73]. When transfected to cultured non-myocyte cells which express no endogenous IF proteins, the mutants with in vitro assembly defects produce dot-like aggregates whereas the mutants that can form IFs in vitro yield a seemingly normal IF network in the cellular context. This corroborates well the in vitro assembly findings. Since all these tested mutants are DRM causing mutants which lead to formation of

aberrant desmin aggregates in myocytes of DRM patients, these in vitro findings suggest that aberrant protein aggregation and disruption of desmin filaments are an intrinsic property to only some of the DRM-linked DES mutants but not to others. The latter may give rise to the disease phenotype only in the natural physiological context of cytoskeletal organization and function in myocytes. For example, pathogenic posttranslational modifications (PTMs) triggered by the mutation might not occur in vitro or in non-natural hosting cells.

Indeed, desmin is subject to a number of known PTMs, including phosphorylation, ADP-ribosylation, and ubiquitination as well as non-enzymatic modifications such as glycation, oxidation and nitration [74]. These PTMs are likely crucial to its conformation and function. Many of the *DES* mutations directly remove or add amino acid residues that can be modified by, for example, phosphorylation (Ser, Thr, Tyr) or ubiquitination (Lys) (Fig. 12.4), which could potentially alter the PTMs of the mutant DES and thereby alter desmin filament assembly/disassembly and/or desmin protein stability under physiological or stress conditions. It was recently reported that phosphorylation of desmin triggers Trim32-mediated ubiquitination and degradation of desmin during muscle atrophy [75]. Increased desmin phosphorylation, which likely promotes desmin filament disassembly, has been reported in DRM [76]. Oxidized and nitrated desmin proteins were also found in affected muscle of DRM patients [77]. In addition, a missense mutation (I451M) at the C-terminus of DES, which is the first *DES* mutation identified in human idiopathic DCM [78], is found to promote proteolytic cleavage at its head domain and abolish its association with the z-disc in mouse hearts [68]. Therefore, DRM *DES* mutations may cause desmin filament disruption in multiple ways.

Similar to what observed in desmin-null mice, myofibril misalignment and mitochondrial dislocation and dysfunction are all seen in desminopathy human muscles and mouse models of desminopathy [67]. Mitochondrial dysfunction and cell death through a mitochondrial pathway are also observed in $CRYAB^{R120G}$-based DRC mouse hearts [79, 80]. This supports the notion that disruption of the desmin IF network contributes to pathogenesis in DRM. Nevertheless, it is not ruled out that gained toxicity from the disease-linked mutant proteins might have actually played a greater role in causing mitochondrial dysfunction and cell death than loss of desmin function.

Overburdened Protein Quality Control in DRM

Like other cells, myocytes especially cardiomyocytes possess multi-layered protein quality control (PQC) mechanisms serving to minimize the level and toxicity of misfolded proteins. First, with the help from chaperones, the cell attempts to unfold and refold a misfolded polypeptide, and if the repairing effort fails, the misfolded protein is then referred to as terminally misfolded protein and targeted for degradation by primarily the ubiquitin-proteasome system (UPS). The UPS is responsible for targeted degradation of most cellular proteins that are either abnormal or normal

but no longer needed. The UPS does so by two main steps: first, covalent attachment of a chain of ubiquitin (Ub) to the substrate protein molecule via a process known as ubiquitination which is catalyzed sequentially by the Ub activating enzyme (E1), Ub conjugating enzyme (E2), and Ub ligase (E3); and second, the ubiquitinated protein will be shuttled to and recognized and degraded by the 26S proteasome, an ATP-dependent multi-units protease complex with its peptidase activity sequestered in the interior chamber of the 20S core subcomplex [81]. When escaped from or overwhelmed the surveillance of chaperones and the UPS, misfolded proteins undergo aberrant aggregation via hydrophobic interaction to give rise to highly active and toxic soluble oligomers and insoluble aggregates. The protein aggregates, which are enriched in ubiquitinated proteins but inaccessible by the proteasome, are generally believed to be degraded by macroautophagy. Macroautophagy (commonly known as autophagy) is a cellular process that sequesters a portion of cytoplasm by forming a double-membraned vacuole, known as an autophagosome; the latter fuses with the lysosome to form the autolysosome where the delivered cytoplasmic content is degraded by lysosomal enzymes [82].

Both *DES* and *CRYAB* are highly expressed genes in skeletal and cardiac myocytes, with their expression being further induced under stress conditions; therefore, one or both alleles of mutated *DES* or *CRYAB* in the genome will result in a considerably high level of mutant proteins, which would conceivably increase PQC burden more dramatically than other low-expressing genes. Indeed, a recent study showed that the total desmin protein level in muscle biopsies from desminopathy humans could be reach a level higher than 3.5-folds of that of the control individuals and the soluble desmin proteins could be increased by ~15-folds in human desminopathic skeletal muscles [50].

The fact that CRYAB^{R120G} causes DRM represents a remarkable real-world illustration for an indispensable role of chaperones in muscle health. Overexpression of wild type CRYAB suppresses aberrant aggregation of DRM-linked mutant desmin in cultured cardiomyocytes and, conversely, expressing CRYAB^{R120G} exacerbates D7-des aggregation and pathogenesis in mouse hearts [83]. Biochemical studies have revealed that the R120G mutation causes CRYAB misfolding, compromises the chaperone function of CRYAB, and alters its interaction with IFs [84, 85]. In mouse hearts overexpressing CRYAB^{R120G}, two types of protein aggregates are observed with electron microscopy. Type I has a homogenous appearance with clear boundaries and is, as revealed by immunogold staining, positive for CRYAB but not desmin; however, type II shows the amorphous appearance of the electron-dense granular-filamentous structures that are similar to the desmin aggregates observed in DRM patients, immunopositive for both desmin and CRYAB [70]. This observation suggests that the formation of desmin aggregates is due to lacking protection of functional CRYAB rather than attraction of misfolded CRYAB^{R120G}. Hence, in the case of CRYAB mutations, both loss of a critical chaperone of desmin and the increased burden to remove misfolded CRYAB^{R120G} are likely in play.

Both myocardial total ubiquitinated proteins and in vitro proteasome peptidase activities are significantly increased in both D7-des and CRYAB^{R120G} transgenic mice, indicative of UPS dysfunction in the heart of the DRC mouse models [86, 87].

To better assess UPS performance in vivo, an inverse UPS function reporter mouse model was created by ubiquitous and constitutive expression of a transgenic modified green fluorescence protein (GFP) with carboxyl fusion of a known ubiquitination signal sequence degron CL1, referred to as GFPdgn. In GFPdgn transgenic mice, an increase in GFPdgn proteins in absence of changes in protein synthesis would indicate a decreased UPS performance and vice versa [88]. Taking advantage of this reporter mouse, myocardial UPS functional insufficiency is revealed in both DRC transgenic mice [86, 87]. It remains to be investigated whether desmin loss-of-function directly impairs UPS function but loss-of-function of CRYAB is unlikely the cause of UPS impairment seen in CRYAB^{R120G} transgenic hearts because knockout of *CRYAB* in GFPdgn transgenic mice via cross-breeding failed to increase myocardial GFPdgn protein levels [86]. Aberrant protein aggregation impairs proteasome function in cultured cells [89]. This provides a reason for UPS function impairment in both D7-des and CRYAB^{R120G} expressing hearts; indeed, inhibition of aberrant aggregation of D7-des or CRYAB^{R120G} by either genetic or pharmacological means markedly attenuated these DRM-linked mutant proteins from impairing UPS performance in cultured cardiomyocytes [86, 87, 90]. These studies provide the first demonstration of UPS impairment by aberrant protein aggregation in intact animals. A major pathogenic role for proteasome functional insufficiency (PFI) in DRC or proteinopathies in general has further been demonstrated by subsequent studies showing that enhancing proteasome function by either genetic or pharmacological means can significantly reduce the prevalence of protein aggregates in cardiomyocytes, slow down disease progression, and delay the premature death of CRYAB^{R120G} mice [91, 92].

Ultrastructural examination of human DRM muscle biopsies shows increased abundance of autophagic vacuoles in the myocytes affected, suggesting that the alternative proteolytic pathway for PQC in the cell, autophagy might be activated in DRM muscles [93]. Indeed, activation of autophagy in cardiomyocytes has been observed in several mouse models of DRC [94, 95]. This activation is compensatory for increased proteolytic stress because genetic suppression of autophagy was shown to exacerbate pathology and disease progression in a CRYAB^{R120G}-based DRC mouse model [94]. Despite of increased autophagic flux, further activation of autophagy by overexpression of Atg7 was shown to protect against the toxicity of CRYAB^{R120G} in both cultured cardiomyocytes and DRC transgenic mice [96, 97]. Taken together, these studies suggest that the autophagosomal-lysosomal pathway is activated but is inadequate and this inadequacy, just like UPS inadequacy, contributes to DRM pathogenesis.

Overburdening PQC is certainly regarded as gained toxicity from DRM-linked mutant proteins. Another example of gained toxicity resulting from CRYAB^{R120G} is redox disturbance, on which conflicting reports exist though. Work from Robbins' group shows that increased oxidative stress derived from mitochondrial malfunction contributes to DRC pathogenesis in CRYAB^{R120G} transgenic mice [80]; however, intriguing data primarily from Benjamin's group support a major pathogenic role for increased reductive stress in the heart of a similar CRYAB^{R120G} mouse model [71, 98, 99]. A unified explanation for such controversy is currently lacking but is

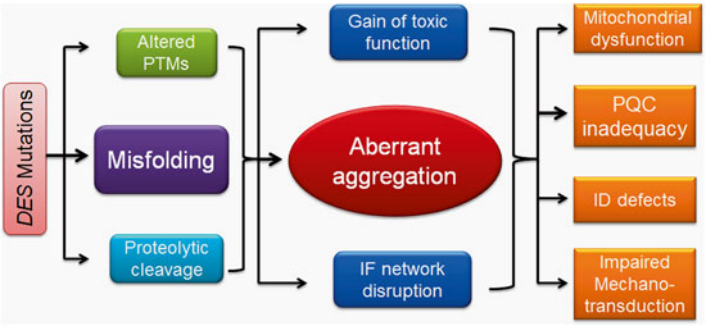

Fig. 12.6 A scheme of pathogenic processes of desminopathy. *PTMs* posttranslational modifications, *IF* intermediate filament, *PQC* protein quality control, *ID* intercalate disc

desperately needed because the completely opposite therapeutic strategies would be otherwise implicated.

In summary, a number of mutually non-exclusive mechanisms are potentially taken by DRM-linked mutations to cause DRM. It is likely that genetic mutations per se or its secondary effects (e.g., proteolytic processing, altered PTMs, etc.) increase the level of toxic desmin proteins in affected myocytes and disrupt the desmin IF network, which in turn either individually or in combination causes PQC inadequacy, mitochondrial dysfunction, intercalated disc defects, and perhaps impaired mechano-transduction. So far, most mechanistic studies support a central role for aberrant protein aggregation (not necessarily the final insoluble aggregates) in DRM pathogenesis (Fig. 12.6).

Experimental Therapeutic Exploration for DRM

The CRYAB^{R120G}-based DRC mouse model not only has been extensively utilized for pathogenic studies but has been facilitating therapeutic exploration as well. Sanbe et al. have reported that preamyloid oligomers (PAO) are increased cardiomyocytes of CRYAB^{R120G}-based DRC mice and voluntary exercise can significantly reduce myocardial PAO and remarkably slow down disease progression and delay premature death of mice in this mouse model [100, 101]. Zheng et al. show that oral administration of high dose of doxycycline leads to reduction of aberrant protein aggregation, ameliorates cardiac pathology, and delays premature death in CRYAB^{R120G}-based DRC mice [102]. Ranek et al. demonstrate that phosphodiesterase five specific inhibitor sildenafil administrated via osmotic minipumps can also reduce myocardial aberrant CRYAB aggregation and slow down disease progression in the CRYAB^{R120G}-based DRC mice through likely PKG activation and thereby priming the proteasome [92, 103]. More recently, McLendon et al. show that histone deacetylase (HDAC) inhibition with SAHA further increases myocardial levels of acetylated tubulin and cardiac autophagic flux, reduces protein

aggregates in cardiomyocytes, and attenuates cardiac dysfunction in the CRYAB^{R120G}-based DRC mice [104].

Currently, no specific therapy is available for treating DRM patients and further understanding DRM pathogenesis and search for more effective measures to intervene DRM or DRC are doubtlessly urgently needed. However, the experimental treatments summarized above not only target known pathogenic factors revealed by basic research but also use clinically readily available drugs or measures, rendering it relatively easier to be translated to the clinic.

Concluding Remarks

It is clear that desmin filaments play an indispensable role in maintaining the structural integrity and mechanical function of muscle tissues after birth but it is also equally certain that function of the desmin IF network remains to be fully understood. Moreover, the pathogenic mechanisms of DRM are far from fully understood and experimental research to target these known mechanisms has just begun to emerge, further effort is warranted. For instance, improving the degradation of the DRM-causing mutant proteins currently is stalled at grossly enhancing the proteasome or autophagy, a thorough understanding of the specific factors that suppress specifically the expression of the mutant gene or increase the targeted degradation of the mutant protein would lead to more specific intervention for the disease. Such effort will be extremely significant because aberrant protein aggregation and inadequate PQC implicated in DRM pathogenesis have also been observed in more common forms of life-threatening human disease, such as neurodegenerative disease and congestive heart failure.

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