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Emery-Dreifuss muscular dystrophy at the nuclear envelope: 10 years on

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Abstract. Emery-Dreifuss muscular dystrophy (EDMD) is a neuromuscular degenerative condition with an associated dilated cardiomyopathy and cardiac conduction defect. It can be inherited in either an X-linked or autosomal manner by mutations in the nuclear proteins emerin and lamin A/C, respectively. Traditionally muscular dystrophies were associated with defects in sarcolemma-associated proteins and, therefore, a nuclear connection suggested the existence of novel signalling pathways associated with this group of diseases. Subsequently, other mutations in the lamin A/C gene were attributed to a range of tissue-specific degenerative conditions, collectively known as the 'laminopathies'. Therefore, any proposed hypothesis underlying the molecular mechanism of EDMD needs to include this anomaly. As we celebrate the 10th anniversary of the identification of emerin as a component of the nuclear envelope, I discuss here the available evidence that currently implicates EDMD as arising from perturbations in myogenic regulatory pathways, causing temporal delays in both cell cycle progression and muscle regeneration.

Keywords. Emerin, Emery-Dreifuss muscular dystrophy, lamin A, laminopathies.

Introduction

Emery-Dreifuss muscular dystrophy (EDMD) gained independent status in 1966 from the more commonly known Duchenne (DMD) and Becker muscular dystrophies (BMD), when it was described by A. E. H. Emery and F. E. Dreifuss as an 'unusual type of benign X-linked muscular dystrophy' [1]. It was classed as 'unusual' because in early adulthood, EDMD patients develop a dilated cardiomyopathy (DCM) with a specific conduction defect, the latter of which is not observed in Duchenne and Becker patients. In 1994, Daniela Toniolo's group identified the X-linked EDMD gene locus at Xq28 (*EMD* or *STA* gene) [2]. The *EMD* gene encodes for a single membrane spanning protein of predicted molecular mass of 29 kDa, which was named emerin. A few years later, two groups reported at the same time that emerin was not only a ubiquitously expressed protein, but also that its subcellular localisation was to the inner nuclear membrane [3, 4]. At the time this was a revolutionary finding; historically muscular dystrophies arose due to defects of proteins associated with the sarcolemma, either directly or via cytoskeletal contacts. Both DMD and BMD arise from disruptions in the gene that encodes for such a protein (dystrophin), which is found on the cytoskeletal face of the sarcolemma in both skeletal and cardiac muscle. Dystrophin binds to both cytoskeletal F-actin and to the integral plasma membrane protein, dystroglycan and together they form part of a multi-molecular complex (dystrophin-glycoprotein complex), linking cytoskeletal components to the extracellular matrix [5] (Fig. 1). This protein complex plays a pivotal role in maintaining the structural framework of muscle. It is easy to see how defects in proteins at this subcellular location would lead to a loss in muscle cell integrity, which over the course of time leads to muscle weakness. But how could a similar

clinical phenotype arise from defects in a nuclear envelope protein? Even more intriguingly, how could defects in emerin generate cardiac conduction defects? The sceptics remained unconvinced that emerin was really localised to the nuclear envelope, until it was reported that the autosomal form of EDMD arose due to defects in the *LMNA* gene [6]. This encodes for the alternatively spliced nuclear lamin A and C intermediate filament proteins, which form a dense filamentous network known as the nuclear lamina underlying the inner nuclear membrane. Shortly afterwards, lamin A/C, was shown to co-immunoprecipitate as part of a novel nuclear envelope protein complex that also included the proteins emerin, lamin B and F-actin [7] Subsequently, a direct interaction between lamin A and recombinant emerin was demonstrated [8]. Thus, the gene products of the X-linked and autosomal forms of EDMD were binding partners. More importantly, a novel macro-protein complex had been identified at the nuclear envelope. The race had begun to unravel the functions of a completely novel signalling mechanism, which, if defective at either of at least two points, results in the muscular dystrophy phenotype.

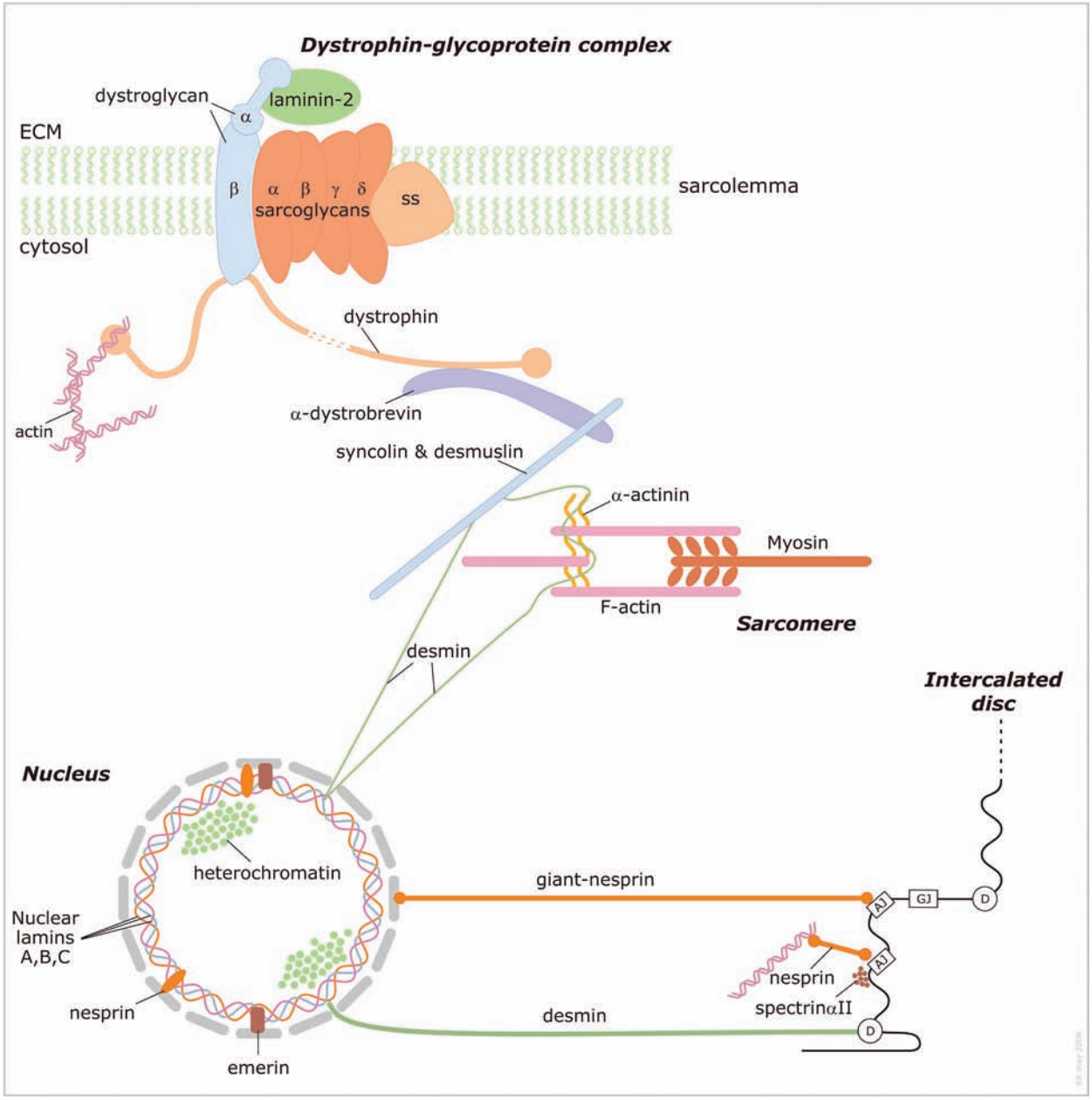

Figure 1. Schematic diagram illustrating possible nuclear envelope/intercalated disc connections in the cardiomyocytes. ECM, extracellular matrix; ss, sarcospan; AJ, adherens junctions; GJ, gap junctions; D, desmosomes. Adapted from [55–57].

Pathology

On the surface EDMD may initially present itself as a mild type of BMD, with a slower progression of muscle wasting and involving different muscles, which span a humeroperoneal distribution. Early contractures at the elbows, ankles and back of the neck are seen before significant muscle weakness [9]. By adult life the mobility of most patients is severely restricted, but patients remain ambulant. It is the cardiomyopathy that is significantly different from DMD/BMD. It can involve combinations of bradyarrhythmia, atrial fibrillation/flutter, supraventricular and ventricular arrhythmias, atrioventricular conduction defects, DCM, non-DCM, restrictive cardiomyopathy and sudden cardiac death (reviewed in [10, 11]). Unlike DMD/BMD, it is the cardiac involvement and not the systematic neuromuscular disorder that places the EDMD patient at risk. Neglected, and it is associated with a substantial risk of sudden cardiac death, even in asymptomatic female carriers. The vast majority of mutations in emerin exhibit the null phenotype, with about 15% expressing modified forms of emerin. However, there is little difference in clinical severity between the two groups of patients and X-EDMD exhibits a fairly constant phenotype [12, 13] (F. Muntoni, personal communication). Unlike emerin, mutations in the lamin A/C gene are generally autosomal dominant missense mutations (A-EDMD), although recessive cases of EDMD are found [14, 15]. Thus, emerin mutations are associated with a loss of function and lamin A/C with a gain or change in function. Furthermore, A-EDMD patients present with a greater variety of, and frequently more severe, clinical phenotypes, than X-EDMD patients. For example, the cardiomyopathy is frequently more severe in A-EDMD than X-EDMD patients, necessitating insertion of a defibrillator rather than a pacemaker [16].

Dystrophic skeletal muscle shows a gradual decline in regenerative capacity with disease progression, which ultimately leads to cell death followed by fibrosis. This is seen in EDMD, although it is somewhat milder than in DMD/BMD patients [17]. Myofibrillar changes observed in EDMD patients include a variation in myofibre size, abnormalities in myonuclear architecture (*e.g.* chromatin condensation), and secondary loss and/or a redistribution of other nuclear antigens [18–20]. Interestingly, only 10% of mytotubes in any one muscle exhibit these structural changes compared with 5% of myotubes in normal individuals, suggesting a low threshold for disease induction. The only significant cardiomyocyte molecular abnormality described concerns the disorganisation of the desmin-lamin B interaction at the nuclear envelope (see below) [21, 22].

The emergence of multiple-emerin-lamin A/C containing protein complexes at the nuclear envelope

In 1996 emerin was the first nuclear envelope protein to be associated with a disease phenotype. Prior to this discovery only a few inner nuclear membrane proteins had been characterised. However, once emerin was identified there was a surge of interest to determine both its binding partners and other nuclear envelope components. As a consequence there are now known to be at least 90 inner nuclear membrane proteins [23] of which 13 have been characterised at the molecular level. Many of these are selectively retained at the nuclear envelope through their interaction with nuclear lamins. This last group includes the lamin B receptor (LBR), lamina-associated polypeptides 1 and 2 (LAP1 and LAP2), emerin, MAN1, SUN-1 and nesprin-1 and -2 isoforms. As a group these proteins share several functional features, including the possession of a large hydrophilic N-terminal domain, which projects into the nucleoplasm and is phosphorylated, one or more transmembrane regions located towards the C terminus of the protein to anchor it to the nuclear membrane in a type II typology (N terminus facing the nucleoplasm) and specific nuclear envelope targeting sequences. In the course of characterising novel inner nuclear membrane proteins, it was noted that emerin shares a homologous N-terminal domain with the LAP2 family members and MAN1, which is termed the 'LEM' domain (for LAP-Emerin-MAN1; [24]) and confers the ability to bind to 'barrier-to-autointegration factor' (BAF), a DNA bridging protein involved in higher-order chromatin function and nuclear membrane assembly. BAF recruits LEM-domain containing proteins to chromatin during nuclear assembly and thus provides a physical link between the nuclear envelope and chromatin. Emerin appears to have an inexhaustible list of binding partners, which currently include the transcriptional repressors GCL (germ cell less) and Btf (death promoting transcriptional repressor), a RNA-splicing associated factor, YT521-B, the inner nuclear membrane isoforms of nesprins-1 and -2, MAN1, nuclear actin, nuclear myosin I, nuclear alpha II spectrin and nuclear lamins A, B and C (reviewed in [25]). Characterisation of their binding sites reveals extensive overlap between binding partners, suggesting emerin is able to exist in several different multi-component protein complexes [25]. Emerin requires both lamin A/C and nesprin-1α to be selectively retained at the nuclear envelope [26, 27]. Since inframe mutations in emerin affect its ability to interact with different binding partners more significantly than reducing its presence at the nuclear envelope [25], it is likely to be the loss of emerin-protein interactions presumably feeding onto a common signalling pathway that gives rise to X-EDMD.

In contrast to emerin, the proteins lamin A and C were first described in 1980 and many functions are attributed

to them, including regulating nuclear growth and shape, DNA replication, chromatin organisation, RNA splicing, cell differentiation, apoptosis, and cell cycle-dependent control of nuclear architecture. Similarly to emerin, lamin A/C has many binding partners, which includes F-actin, MAN1, emerin, LBR, retinoblastoma protein (Rb, a tumour suppressor), protein phosphatase 2A, SREBP-1c, nesprin-1, histones, Young Arrest (YA), nuclear titin isoforms, LAP1 and LAP2α and β, BAF, MOK2 (RNA and DNA binding protein), Lco-1 and components of RNA pol II and DNA-replication complexes (reviewed in [28]). Lamin A/C and emerin can, therefore, exist in the same as well as different nuclear envelope-associated complexes, suggesting that they possess at least some functions that are independent of one another.

The 'laminopathies'

Before discussing the possible molecular mechanisms underlying the pathology of EDMD, we need to take into account that mutations in the *LMNA* gene can additionally cause up to 11 other clinically distinct tissue-specific degenerative diseases, which are collectively termed the 'laminopathies' and are briefly described in Table 1. The laminopathies can be broadly split into two groups: (i) neuromuscular disorders affecting directly the skeletal muscle, cardiac muscle and peripheral nervous system (*e.g.* EDMD, DCM, Limb-girdle muscular dystrophy 1B (LGMD1B) [10]; and (ii) partial lipodystrophy syndromes with or without developmental abnormalities and premature ageing [*e.g.* mandibuloacral dysplasia and Hutchison-Gilford Progeria Syndrome (HGPS)] [29]. As the *LMNA* patient database has grown (http://www.dmd.nl/nmdb/index.php?select_db=LMNA and www.umd.be.2000), no clear genotype-phenotype correlation has become apparent, although mutations giving rise to the second group of diseases tend to cluster towards the C-terminal region of the lamin A/C protein. However, this classification is rather misleading since there is considerable overlap in phenotype between the different laminopathies and within families the same *LMNA* mutation can lead to the expression of a different clinical phenotype, including non-penetrance. This observation suggests the genetic background of the individual is important. There is already some evidence for this. In two A-EDMD patients exhibiting a severe form of the disease, the severity of the phenotype was attributed to digenic inheritance [21, 30]. Similarly, this has also been shown for a severely affected LGMD1B patient [31]. It may be that as a group, the laminopathies have a tendency towards polygenic inheritance to explain this phenotypic variation. Furthermore, 19 single nucleotide polymorphisms (SNPs) are associated with the *LMNA* gene in the general population and some are found in patients (G. Bonne and P. Richard, personal communication). Currently, these have not been evaluated for how they may influence the disease penetrance. In addition, phase 1 of the International HapMap project was recently completed (www.hapmap.org/groups.html), which analyses patterns of common variation in the human genome, mainly through mapping SNPs. Thus, future SNP correlations could be extended to a genome-wide search, which would pin-point possible modifying genes that additionally influence the laminopathy disease penetrance. Therefore, in proposing a molecular mechanism for EDMD we need to take into account the more variable penetrance of the disease phenotype observed in A-EDMD then in X-EDMD patients.

Table 1. Classification of diseases due to mutations in the *LMNA* gene. Adapted from [10] and Leiden muscular dystrophy website http://www.dmd.nl/lmna_home.html. Incidence of X-EDMD is 1:1 million and the laminopathies 1:200 000 (Fiona Norwood, Katie Bushby, Francesco Muntoni personal communication).

Laminopathy type	Inheritance	Acronym	OMIM
(1) Striated muscle involvement			
Emery Dreifuss muscular dystrophy	AD AR	AD-EDMD-2 AR-EDMD-3	181350 604929
Dilated cardiomyopathy with conduction system disease	AD	DCM-1A	115200
Limb-girdle muscular dystrophy with cardiac involvement	AD	LGMD1B	159001
Charcot-Marie-Tooth, axonal neuropathy	AR AD	CMT-2B1 AD-CMT-2	605588
(2) Lipodystrophy and premature ageing			
Familial partial Dunnigan type lipodystrophy	AD	FPLD	151660
Mandibuloacral dysplasia I	AR	MAD-1	248370
Hutchison Gilford progeria syndrome	AD	HGPS	176670
Lipoatrophy with diabetes, hypertrophic cardiomyopathy, hepatic steatosis, leukomelanodermic papules	AD	LDHPC	608056
Cardiocutaneous progeria syndrome	AD	CCPS	277700
Restrictive dermopathy	AD	RD	275210

Possible molecular mechanisms underlying the EDMD phenotype

There is currently experimental evidence in support of two molecular mechanisms to explain the EDMD pathology. These are the nuclear fragility and gene expression hypotheses, which are not mutually exclusive [32]. The physical defects associated with the myonuclei from both X- and A-EDMD patients imply they would be sensitive to mechanical stress. Indeed, *Lmna*$^{-/-}$ but not *Emd*$^{-}$

$^{/-}$ mouse embryonic fibroblasts exhibit impaired nuclear mechanics in response to strain [33–35], although both cell types exhibit impaired expression of two mechanosensitive genes. The gene expression model proposes that perturbing interactions within the emerin-lamin A/C complex disrupt interactions at specific chromatin sites and/or with tissue-specific transcriptional regulators, leading to downstream effects on chromatin structure or gene expression in a specific tissue. If this is true, then the pathophysiology observed in any of the laminopathies may not just be a reflection on the primary molecular defect observed. The model would also provide a satisfactory explanation for the multiplicity of tissue-specific diseases that mutations in lamin A/C can cause, and also how the ubiquitously expressed emerin only affects striated muscle function. In addition, it explains why the *Emd*$^{-/-}$ cells exhibited a change in gene expression under mechanostress. Indeed, changes in downstream gene expression have recently been reported in fibroblasts and myoblasts from both X- and A-EDMD patients and from animal models of EDMD [33, 34, 36–38]. Bioinformatic array analysis on either regenerating muscle isolated from emerin null mice [37] or muscle biopsies isolated from both X-EDMD and A-EDMD patients [38] demonstrated perturbations in cell cycle parameters and delayed myogenic differentiation. This phenotype was linked to an upregulation of retinoblastoma (Rb1) and Myo D genes as a possible compensatory mechanism to overcome a block in the myoblast/myotube transition. These data suggest that interactions between Rb1/Myo D and nuclear envelope components are disrupted at the point of exit from the cell cycle. Interestingly, this finding was not observed on regenerating muscle isolated from the lamin A/C null mouse [37], suggesting that the lamin A/C null mouse may be quite mechanistically different to the dominant missense mutations associated with A-EDMD patients. However, conflicting evidence has been published to suggest that immortalised myoblasts from the lamin A/C null mice have an impaired differentiation potential [39]. Furthermore, *Lmna*$^{-/-}$ fibroblasts have also been reported to have an increased proliferative potential compared with wild-type cells, which is likely to arise from defective TGF-β1-Rb-SMAD signalling [40]. A-type lamins are known to protect Rb from proteasomal degradation and in lamin A/C null cells total Rb levels drop, perturbing the cell cycle [41]. These recent findings are consistent with much earlier reports of cell cycle-dependent aberrant emerin phosphorylation and extended cell cycle length in X-EDMD cells [42, 43] and that a functional lamin-scaffold needs to be constructed for the correct regulation of Rb pathways involved in skeletal muscle differentiation [44]. Taken together these experiments provide a functional link between structural components of the nucleus and pathways concerned in exiting the cell cycle to allow tissue-specific cell differentiation to occur. These data support a previously proposed theory that mutations in emerin and lamin A/C must selectively effect the differentiation, maintenance, repair or regulation of cells in the mesenchymal stem cell lineage (which gives rise to muscle, fat and bone tissue), by affecting gene expression in this lineage [32]. However, associating EDMD with irregularities in myogenic regulatory genes is a novel concept, since historically these genes are associated with abnormal regulation of skeletal muscle regeneration, not to an effect on progressive muscle wasting or weakness, as is seen in muscular dystrophies [45]. Therefore perhaps emerin-lamin A/C mutations giving rise to EDMD cause a temporal delay in muscle regeneration, in which case it is muscle maintenance and not its formation that is at fault. Alternatively, myogenic factors may have more varied functions than originally thought.

We also need to explain how the EDMD pathology fits in with the other laminopathies. Perhaps the laminopathies lie on a sliding scale of premature ageing syndromes, with EDMD at the mild end of the spectrum and HGPS at the other end. The features of cells from X-EDMD and laminopathy patients is consistent with an ageing link, *i.e*. disrupted nuclear structure/chromatin perturbations, decreased cellular proliferation, reduced tissue repair mechanisms and non-maintenance of a differentiated state. Evidence for the last feature has been presented above for EDMD [37, 38] but can be expanded to include the other laminopathies. Every cell type possesses individualistic differentiation programmes, with different molecular players, which will vary in their interactions with the common molecular player, namely lamin A/C. It is this variation which generates the family of lamin A/C-linked diseases. Although chromosome positioning is also affected in a number of fibroblasts derived from patients with a range of lamin mutations, as well as in fibroblasts with a heterozygous emerin mutation (K. Meaburn and J. Bridger, personal communication), patients with lamin A and homozygous emerin mutations show no major alterations to the genome organisation [46, 47]. In addition, the normal ageing process is associated with loss of muscle mass and bone density and fat redistribution, all of which are found in one laminopathy or another [48]. In this context, *LMNA* mutations will generate a spectrum of clinical phenotypes, which are determined by the position of the mutation in the gene and the individual's genetic background.

Future perspectives

There remain many unanswered questions concerning the aetiology of EDMD. To begin with, only 40% of patients clinically diagnosed with EDMD possess either emerin or lamin A/C mutations, suggesting that other components of the emerin-lamin A/C protein complexes

are candidate genes for EDMD [12]. Secondly there are no published reports examining the tissue specificity of the emerin-binding partner interactions, which are central to the gene expression hypothesis. Thirdly, the proposed molecular mechanisms do not translate easily to explain the conduction defect. There is no loss of atrial musculature or any morphological abnormalities attributed directly to the conduction tissue [49]. Since the insertion of a pacemaker or defibrillator device can alleviate the dysrhythmias, it suggests that the cardiac contractile function is not affected *per se* – but that the disease selectively targets the conduction tissue. This would suggest that the small amount of fibrosis seen in EDMD patients (small compared with DMD patients) does not impair electrical coupling. Fourthly, there are several unconfirmed reports of emerin localising to structures localised outside the nuclear envelope, notably the intercalated discs [50], tubular aggregates derived from the sarcoplasmic reticulum [51], platelets [52] and spreading initiation centres in cell migration [53]. Interestingly, one of emerin's nuclear binding partners, alpha II spectrin has also recently been reported to associate with specific regions of the intercalated discs [54]. However, it is difficult to reconcile which known intracellular trafficking pathway would be able to re-direct a protein with nuclear targeting motifs to other subcellular localisations. In addition, lamin A/C is exclusively nuclear associated. Alternatively, the conduction defect could arise as a result of the weakening of the desmin-lamin B [21, 22] interaction seen at the nuclear envelope of cardiomyocytes from *Lmna*$^{-/-}$ mice. The cytoplasmic end of desmin interacts directly with both desmosomes of the intercalated discs and with the intermediate filament proteins syncolin and desmuslin (also known as synemin) at the sarcolemma-cytoskeletal network (see Fig. 1; [55]). These in turn both bind directly to dystrobrevin, a component of the dystrophin-glycoprotein complex. Thus, there is a continuous filamentous network radiating from the nuclear envelope to the sarcolemma. Similarly, the giant nesprin-1 and-2 isoforms are large enough to perform the same function [56, 57]. These could link the emerin-lamin A complex at the nuclear envelope to both the sarcolemma-associated dystrophin complex and to the intercalated discs. A primary defect in emerin/lamin A/C could therefore indirectly affect the function of remote structures to explain the observed EDMD phenotype. Indeed, the actin, vimentin and tubulin-based cytoskeletal filamentous networks exhibit disturbed organisation in *Lmna*$^{-/-}$ mouse embryonic fibroblasts [35]. It remains to be seen whether this explains the pathology observed in EDMD patients.

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