Lipodystrophic laminopathy: Lamin A mutation relaxes chromatin architecture to impair adipogenesis

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The familial partial Dunnigan lipodystrophy, characterized by subcutaneous fat loss, is frequently caused by an R482W mutation in lamin A. In this issue, Oldenburg et al. (2017. *J. Cell Biol.* https://doi.org/10.1083/jcb.201701043) demonstrate that this mutation impairs the ability of lamin A to repress the anti-adipogenic miR-335, providing a potential molecular mechanism for the disease.

The nuclear lamina is a filamentous protein meshwork that is located at the nucleoplasmic surface of the nuclear envelope, attached to integral nuclear membrane proteins and nuclear pore complexes. It is primarily composed of lamins, which are type-V intermediate filament proteins that can be further classified into two distinct types (A- and B-types) based on their sequence homology and tissue-specific expression. The alternatively spliced variants, lamin A and C, encoded by the *LMNA* gene, represent the major A-type lamins. Whereas the B-type lamins are constitutively expressed in all cell types, the A-type lamins are mainly expressed in differentiated cells. In addition to the lamina, lamin A/C can also localize in the nucleoplasm in lamina-independent complexes, whereas the B-type lamins only exist at the nuclear periphery (Gruenbaum and Foisner, 2015). The filamentous structure of the lamin network, together with dynamic changes in the levels of the different isoforms, allows the nuclear lamina to bear and adapt to a wide range of mechanical insults, such as compression or stretching, which cells and tissues continuously experience. Consistently, impairment in the structure and mechanical resilience of the lamina negatively impacts nuclear architecture and genomic integrity, which in the long run likely has substantial effects on cell fate and behavior (Miroshnikova et al., 2017).

In addition to maintaining the shape, stiffness, and structural integrity of the nucleus and the genome, the nuclear lamina is involved in several gene regulatory and signaling functions. It has been reported to anchor and position nuclear pores, as well as to regulate cell cycle entry and progression, nuclear disassembly in mitosis, DNA replication and repair, higher-order chromatin organization, gene positioning, and gene expression (Gruenbaum and Foisner, 2015). As such, nuclear lamins and other lamina-associated proteins are important mediators of fundamental processes such as tissue morphogenesis and maintenance. The diverse roles of lamins in nuclear functions stem from their ability to interact with DNA, histones, and chromatin at specific sites referred to as lamin-associated domains (LADs), which orchestrate global chromatin organization and epigenetic silencing states (Kind and van Steensel, 2014). LADs contain mostly transcriptionally inactive genes and intergenic regions and are enriched in the repressive histone marks H3K9me2/3 and H3K27me3. Several studies have reported that anchoring genes to the lamina correlates with gene repression, indicating that tethering genomic loci to the nuclear periphery could be a mechanism to stably repress genes during differentiation (Kind and van Steensel, 2014). Although LADs were initially reported to localize at the nuclear periphery, single-cell analyses have revealed that they are in fact stochastically redistributed during the cell cycle between the lamina and the nuclear interior, where they tend to localize around nucleoli (Kind and van Steensel, 2014). This is consistent with recent data indicating that the majority of A-type lamins in the nuclear interior exist in a mobile state, which is distinct from the insoluble, filamentous structure of the nuclear periphery (Turgay et al., 2017). However, the precise roles and functions of this intranuclear pool of lamins and the mechanisms of gene silencing at this site are unclear.

Given their importance in regulating nuclear mechanics, chromatin organization, and gene expression, it is not surprising that mutations in lamin proteins lead to human disease. A growing number of diseases, commonly referred to as laminopathies, have been attributed to mutations in lamins or lamin-associated proteins after the first implication of mutations in the *LMNA* in the Emery-Dreifuss muscular dystrophy discovered in the late 1990s (Jacob and Garg, 2006; Gruenbaum and Foisner, 2015). As laminopathies currently lack specific, curative treatments, understanding the mechanisms by which these mutations compromise the structural and functional properties of lamins is of importance. With over 500 reported mutations to date, mutations in the *LMNA* gene are by far the most common, and thus responsible for the majority of laminopathies. Patients with laminopathies commonly share overlapping pathologies, which can be categorized under four major subgroups: lipodystrophies, skeletal muscle disorders, peripheral neuropathies, and premature aging (Gruenbaum and Foisner, 2015). In this issue, Oldenburg et al. provide a molecular mechanism for how laminopathies might cause lipodystrophies with the demonstration that a mutation in lamin A associated with lipodystrophy results in up-regulation of the anti-adipogenic miR-355, thereby impairing expression of adipogenic genes.

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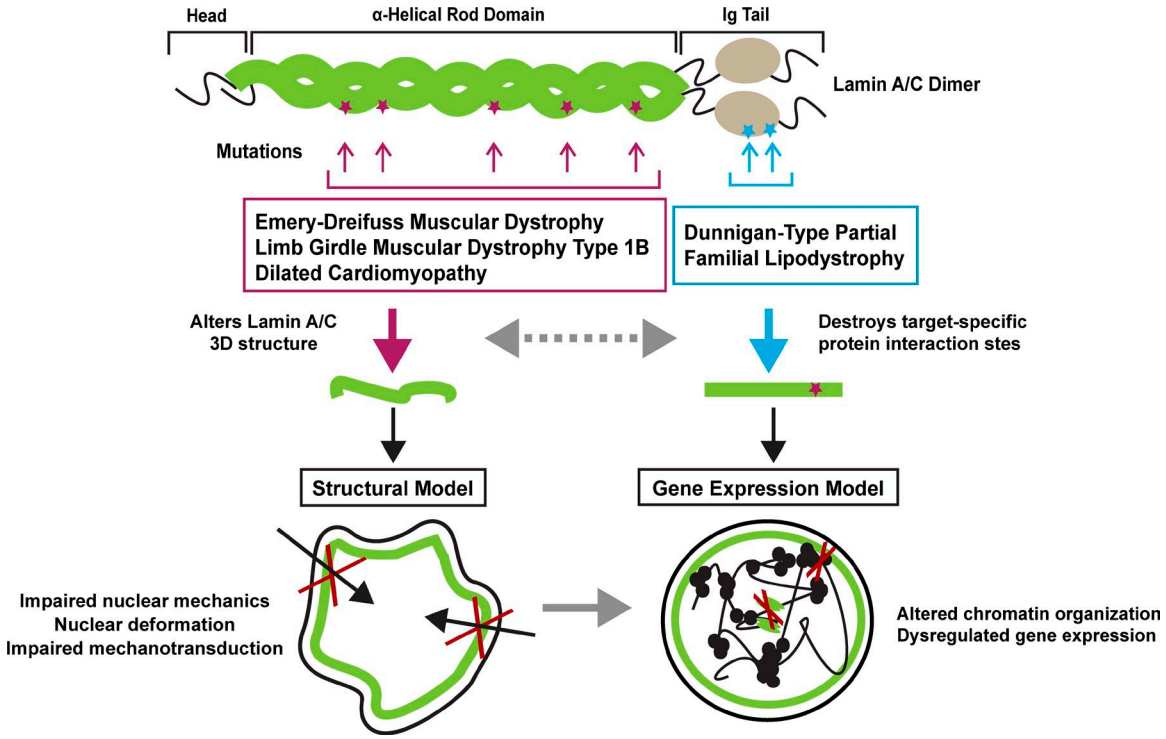

Figure 1. **Structural and gene expression models in laminopathies.** Lamins are dimers composed of a central α-helical rod domain flanked by a short N-terminal (head) domain and a globular C-terminal (tail) domain with an Ig motif. Mutations associated with muscular dystrophies (Emery-Dreifuss muscular dystrophy, limb girdle muscular dystrophy type 1B, and dilated cardiomyopathy) are linked primarily to the helical rod domain, and to a lesser extent to the C-terminal domain. These mutations reside in critical positions that disrupt lamin A/C structure, impairing nuclear integrity and the ability of the nucleus to respond to mechanical cues (structural model). Mutations linked to the Dunnigan-type partial familial lipodystrophy, in contrast, reside within the C-terminal tail and most likely do not alter the 3D lamin structure, but rather disrupt target-specific protein interaction sites, thereby inducing specific changes in chromatin organization and/or gene expression (gene expression model). The two models most likely interplay in the pathogenesis of the different laminopathies, especially considering that nuclear integrity and altered mechanics are likely to impact chromatin organization, and several mutations are likely to have effects both on protein structure and interactions (gray arrows).

Two main mechanisms have been proposed to generally explain how laminopathy-causing mutations mediate the observed phenotypes: the mechanical disease model, or structural model, and the gene expression model (Fig. 1). The structural model proposes that lamin mutations impair the ability of the nuclear lamina to maintain nuclear integrity and to transmit external mechanical signals into the nucleus. The gene expression model, however, suggests that lamin mutations impair the capacity of lamins to interact with chromatin and/or transcriptional regulators central to cellular fate (Gruenbaum and Foisner, 2015). Both of these mechanisms are most likely relevant and act in parallel, or even in interplay, but they might also have distinct and differential contributions in different tissues, which would in part explain the tissue-specific nature of laminopathies (Lammerding et al., 2004). In addition, specific mutations might selectively impair only a subset or even a single specific function of lamin, underlining the need for careful mechanistic dissection of the individual mutations (Fig. 1).

The familial partial Dunnigan lipodystrophy (FPLD2) is an inherited, autosomal-dominant disorder that is commonly caused by a heterozygous R482W mutation in the *LMNA* gene (Jacob and Garg, 2006). Patients with FPLD2 have an abnormal distribution of adipose tissue that is lost and redistributed away from the extremities and trunk around puberty. In addition, these patients typically suffer from metabolic disorders (Gruenbaum and Foisner, 2015). Examining the lamin A protein structure indicates that, whereas *LMNA* mutations that cause muscular dystrophies probably destabilize lamin protein structure, mutations that give rise to FPLD are clustered within a small area of the lamin A/C tail and most likely do not severely disrupt the Ig-like structure of this C-terminal domain (Fig. 1; Gruenbaum and Foisner, 2015). This indicates that the FPLD2-causing mutations are unlikely to give rise to nuclear fragility and therefore probably cause disease by disrupting gene regulation. However, the precise mechanisms of this regulation and the underlying cause for these tissue-specific manifestations are poorly understood.

In their attempt to identify defects in gene regulatory networks underlying FPLD2, Oldenburg et al. (2017) set out to investigate the microRNA miR-335. This microRNA has recently been reported to inhibit proliferation, migration and the differentiation potential of human mesenchymal stem cells into the osteogenic and adipogenic lineage, thereby highlighting the importance of low levels of miR-335 for osteogenic and adipogenic differentiation (Tomé et al., 2011). To assess whether miR-335 plays a role in the etiology of the lipodystrophic laminopathy FPLD2, Oldenburg et al. (2017) examined the expression levels of miR-335 in FPLD2 and observed increased levels of miR-335 in fibroblasts from FPLD2 patients with the LMNA(R482W) mutation when compared with WT fibroblasts. A similar effect was observed in human adipose stem cells (ASCs) expressing the mutant LMNA(R482W). Interestingly, the induction of adipogenesis resulted in a substantial decrease in the miR-335 levels in ASCs, whereas LMNA(R482W) ASCs

failed to down-regulate the anti-adipogenic miR. This lead the authors to hypothesize that these sustained miR-335 levels in LMNA(R482W) ASCs might have an inhibitory effect on adipogenesis. Indeed, a selective impairment in the expression of several early and late adipocyte genes (*PPARG*, *CEBPA*, *FABP4*, *KLF5*, and *KLF15*) and the lipid droplet marker protein, perilipin, was observed upon the adipogenic induction of LMNA(R482W) ASCs. This could be rescued by cotransfection with the miR-335 hairpin inhibitor, 335-i, highlighting the specificity of the defect. Whether these changes in gene expression are sufficient to attenuate or impair adipogenesis in the FPLD2 patients remains open for future studies.

Interestingly, the gene encoding for miR-335 lies specifically within intron 2 of the *MEST* gene, which according to a ChIP-seq performed earlier by the same group, lies between two *LMNA* LAD regions (Rønningen et al., 2015). The inter-LAD region encompassing the MEST/miR-335 locus is itself also associated with lamin A. However, the authors found that even though this is the case, very low levels of lamin A were detected in undifferentiated native ASCs in the promoter proximal region upstream of the miR-335 transcription start site. Upon differentiation, a substantial increase in the levels of lamin A at the same site was observed, paralleled with a corresponding decrease in miR-335 levels. This increase failed to occur in the LMNA(R482W) mutant cells, indicating that the mutation perturbs this interaction. Surprisingly, although adipogenic differentiation induced a repositioning of the MEST/miR-335 locus within the nucleus, a process impaired in the LMNA(R482W) mutant cells, this relocalization did not drive the locus to the nuclear periphery as one would expect. This indicates that lamin A interacts with and represses this locus within the nuclear interior, consistent with previously observed lamin A/C pools at this site and the observed frequency of LADs occurring at locations outside the lamina (Kind and van Steensel, 2014; Turgay et al., 2017). How these interactions occur and are regulated and how exactly the heterozygous LMNA(R482W) mutation induces a dominant-negative effect in this repositioning remain interesting and important aspects for future work.

Consistent with the repressive function of the lamin A–MEST/miR-335 locus interaction, the authors observed that whereas miR-335 promoter and enhancer elements carry similar levels of the silencing H3K27me3 histone mark in undifferentiated ASCs expressing WT LMNA or LMNA(R482W), two enhancers in the mutants already carry acetylated H3K27, indicative of aberrant activation. Furthermore, as expected, differentiation elicits H3K27me3 accumulation at proximal and distal regulatory elements in cells expressing WT LMNA, whereas these sites are acetylated in R482W mutant cells, consistent with impaired silencing. The authors then demonstrated that this distal enhancer shows increased proximity to the miR-335 promoter only in the mutant cells, indicating a conformational change in this locus triggered by the LMNA(R482W) mutation.

Collectively, these findings highlight the importance of lamin A in imposing a repressive chromatin state at the MEST/miR-335 locus during the adipogenic differentiation of ASCs, which in turn ensures suppression of the anti-adipogenic miR-335 to permit expression of adipogenic genes. The model put forward by the results suggests that the heterozygous R482W mutation in the *LMNA* gene exerts a dominant-negative function to impair the ability of lamin A to interact with and silence the MEST/miR-335 locus through regulation of specific chromatin rearrangements and epigenetic changes at the level of enhancer–promoter looping, resulting in the high levels of miR-335 observed in FPLD2.

A key next step will be to address the contribution of this pathway in adipogenesis in vivo and most importantly in the disease phenotype. In addition, it will be interesting to unravel how widespread the effect of this mutation on enhancers is. Multiple other molecular changes caused by this specific LMNA(R482W) mutation have been described, including misregulation of other key adipogenic genes such as *SREBP* (Vadrot et al., 2015). Whether these mechanisms are independent of each other or caused by a widespread defect in enhancer looping needs to be addressed. An interesting follow up experiment will be to analyze changes in LADs, H3K27me3, and H3K27Ac genome wide and assay for global changes in enhancer–promoter looping in the LMNA(R482W) cells. The presence of widespread defects would point to a role of the lamin network in regulating promoter–enhancer interactions, possibly through a general constraining function to restrict chromatin mobility.

Given that the FPLD2 patients display a redistribution rather than a complete loss of adipose tissue, it will further be important to examine this deregulation of adipogenesis in respect to the precise localization of the adipose tissue and thus source of ASCs or preadipocytes. Finally, even though the mutated *LMNA* gene, in this case R482W, is expressed in almost all differentiated cells, it exerts a very tissue-specific effect on gene regulation. How such a ubiquitous protein is capable of regulating chromatin structure at distinct adipose tissue–specific gene loci remains an intriguing open question.

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