A novel interaction between lamin A and SREBP1: implications for partial lipodystrophy and other laminopathies

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The gene encoding nuclear lamins A and C is mutated in at least three inherited disorders. Two of these, Emery–Dreifuss muscular dystrophy (EDMD-AD) and a form of dilated cardiomyopathy (CMD1A), involve muscle defects, and the other, familial partial lipodystrophy (FPLD), involves loss of subcutaneous adipose tissue. Mutations causing FPLD, in contrast to those causing muscle disorders, are tightly clustered within the C-terminal domain of lamin A/C. We investigated the expression and subcellular localization of FPLD lamin A mutants and found no abnormalities. We therefore set out to identify proteins interacting with the C-terminal domain of lamin A by screening a mouse 3T3-L1 adipocyte library in a yeast two-hybrid interaction screen. Using this approach, the adipocyte differentiation factor, sterol response element binding protein 1 (SREBP1) was identified as a novel lamin A interactor. In vitro glutathione S-transferase pull-down and in vivo co-immunoprecipitation studies confirmed an interaction between lamin A and both SREBP1a and 1c. A binding site for lamin A was identified in the N-terminal transcription factor domain of SREBP1, between residues 227 and 487. The binding of lamin A to SREBP1 was noticeably reduced by FPLD mutations. Interestingly, one EDMD-AD mutation also interfered with the interaction between lamin A and SREBP1. Whilst the physiological relevance of this interaction has yet to be elucidated, these data raise the intriguing possibility that fat loss seen in laminopathies may be caused, at least in part, by reduced binding of the adipocyte differentiation factor SREBP1 to lamin A.

INTRODUCTION

Nuclear lamins are members of the intermediate filament family of proteins and are the major component of the nuclear lamina, a fibrous network underlying the inner face of the nuclear envelope (1). Lamins may be classified into two types, A and B, according to their sequence and biochemical properties. B-type lamins are ubiquitously expressed at all stages of development, whereas A-type lamins (lamins A and C) are expressed exclusively in differentiated tissues (2). Lamins are composed of a short N-terminal head domain, followed by a coiled-coil rod domain and a globular C-terminal domain. The proteins form dimers, via their coiled-coil domains, which then polymerize to form the nuclear lamina (3) through interactions with several inner nuclear membrane proteins (reviewed in 4). The lamina is thought to maintain nuclear structure and integrity and contribute to the regulation of chromatin organization by attachment of mainly inactive heterochromatin to the nuclear periphery (5).

Lamins are also present within the nucleoplasm (6) and have been shown to be components of the nuclear matrix (7). Accumulating evidence now suggests that nuclear architecture is far more complex than originally thought, with spatial organization of transcription and DNA replication at discrete sites (8). This is achieved through binding of both the chromatin and the transcription, splicing and replication complexes to the nuclear matrix (9,10). Interestingly, a number of transcription factors associate with the nuclear matrix (11–13), each of which exhibits a distinct subnuclear distribution. Lamins co-localize with sites of DNA replication (14) and with splicing factors (15) and interact with the retinoblastoma gene product (Rb), a transcriptional repressor and tumour suppressor (16). In addition to their role in nuclear stability, lamins are therefore likely to play an important role in the regulation of gene expression and DNA replication, perhaps by providing a scaffold for the attachment and stabilization of multi-subunit protein complexes.

Lamins A and C are products of a single gene, LMNA, produced by alternative splicing at its 3′ end, resulting in proteins of 664 and 572 amino acids, respectively (17,18). Recently, three autosomal dominant disorders have been shown to be associated with mutations in the LMNA gene, two of which specifically target striated muscle tissue: Emery–Dreifuss muscular dystrophy (EDMD-AD) (19) and dilated cardiomyopathy with conduction system disease (CMD1A) (20). Whereas CMD1A involves only cardiac defects, EDMD-AD is characterized by progressive muscle wasting from specific sites of the body, together with cardiac conduction defects. On the other hand, the third disorder associated with LMNA...
mutation (21,22), familial partial lipodystrophy (FPLD), specifically affects subcutaneous adipose tissue and is invariably associated with insulin resistance, diabetes and hyperlipidaemia (23,24). Although at first sight there appears to be no overlap in the clinical phenotype of patients suffering from myopathic and lipodystrophic disease, evidence is now emerging that there may be some features in common. In particular, some EDMD-AD patients exhibit lipodystrophy (F.Muntoni, personal communication), which is supported by the finding that LMNA knock-out mice, in addition to displaying the features of EDMD, also lack subcutaneous adipose tissue (25).

The phenotypic differences of the laminopathies are somewhat reflected in the distribution of mutations in LMNA. Mutations causing EDMD-AD are distributed throughout the gene, whereas those resulting in CMD1A are mainly clustered within the coiled-coil domain, suggesting that the latter may act by disrupting dimerization or subsequent polymerization of lamin A/C. In sharp contrast, the mutations identified to date in FPLD patients are found exclusively within exons encoding the C-terminal domain of lamin A/C and, more specifically, are restricted to just a few codons in exons 8 and 11 (22,26,27).

Since laminas A and C are widely expressed in adult tissues, the reason why mutations in LMNA should only affect muscle and adipose tissues remains obscure. Several hypotheses have been proposed (28,29). Given the unique contractile forces exerted upon muscle cells, these cells may be more sensitive to the nuclear fragility predicted to be caused by mutations in laminas. Alternatively, mutations in lamin A/C may result in changes in tissue-specific gene expression by altering chromatin structure or the assembly/localization of components of transcription complexes.

A crystal structure of the C-terminal domain of lamin A has recently been solved (S. Shoelson, personal communication). This reveals that the highly clustered mutations found in FPLD patients lie on one face of the protein. Based on this observation, we predicted that FPLD may be caused by interference of an interaction of lamin A/C at this interface with a protein required for adipocyte differentiation. To identify adipocyte-specific lamin A/C interacting proteins, we performed a yeast two-hybrid interaction screen of a mouse 3T3-L1 adipocyte cDNA library (31), using the C-terminal domain of mouse lamin A as a bait. Yeast strain PJ69-4a (32) was transformed with pGBDU-mLMNA379, containing mouse lamin A residues 379–664 fused to the GAL4 DNA binding domain (DBD). The resultant strain was then transformed with 3T3-L1 library DNA, contained within the GAL4 activation domain vector pGADGH and 20 positive clones identified.

Sequencing of the 20 cDNA inserts revealed that three clones contained the C-terminus of mouse lamin A; two clones were identical and extended from residue 253 whilst the third extended from residue 280. These cDNAs encoded the whole of the C-terminal domain, together with part of the coiled-coil domain.

**Lamin A interacts with SREBP1 and SREBP2**

Six of the other lamin A-interacting clones encoded mouse SREBP1 and comprised five independent cDNAs with N-terminal truncations at amino acids 54, 63, 126, 130 (two clones) and 220, respectively (Fig. 2). In addition to SREBP1, one clone encoding mouse SREBP2 was isolated from the two-hybrid screen and contained an almost complete cDNA, lacking only the first four amino acids of the protein (Fig. 2).

SREBP1 is a dual specificity transcription factor of the basic-helix–loop–helix–leucine zipper (bHLH-Zip) family that has independently been shown to be involved in both the regulation of cholesterol biosynthesis (33) and in adipogenesis, including the expression of genes involved in fatty acid metabolism (34,35). This is achieved by its ability to bind to upstream sterol response element and E-box sequences,
respectively. SREBP2, on the other hand, although able to recognize both types of enhancer, appears to act mainly to stimulate cholesterol biosynthesis (36). SREBPs have a tripartite structure, composed of an N-terminal transcription factor domain and C-terminal regulatory domain, separated by two transmembrane domains (Fig. 2). SREBP1 exists as two isoforms, SREBP1a and SREBP1c, produced by alternative splicing of the first exon, resulting in unique sequences of 29 and five residues, respectively, at their N-termini. Since none of the clones identified in the two-hybrid screen extended to the region of alternative splicing at the 5′ end of the gene, this suggests that both SREBP1a and SREBP1c are capable of interaction with lamin A. In addition, the extent of the N-terminal truncations indicate that the domain involved in interaction with lamin A lies downstream of amino acid 220, a region which includes the bHLH-Zip motif, the two transmembrane domains and regulatory C-terminus, but lacks the acidic N-terminal activation domain of the protein.

The identification of both SREBP1 and SREBP2 in this library screen suggests that they are bona fide lamin A interacting proteins. However, given that SREBP1 is thought to be the relevant isoform in adipogenesis, we limited further investigations to this protein.

Confirmation of the lamin A-SREBP1 interaction by in vitro and in vivo binding assays

As a preliminary step towards confirmation of the lamin A-SREBP1 interaction, the human homologues were used in a direct yeast two-hybrid interaction assay. Human lamin A (residues 389–664) was fused to GAL4-DBD, and human SREBP1a was fused to GAL4-AD. Co-transformation of these constructs and subsequent reporter gene assays supported the interaction of the mouse proteins (data not shown). All further experiments were therefore performed using the human proteins.

As an independent means of verification of the interaction, an in vitro GST binding assay was performed. GST and a GST-lamin A389 fusion protein were expressed in Escherichia coli BL21 and immobilized on glutathione–Sepharose beads.
Radiolabelled, \textit{in vitro}-translated SREBP1\textsubscript{a} was added to the beads and, following washing, was found to bind to GST-lamin A but not GST alone (Fig. 3A, lanes 2 and 3). In the same way, dimerization of the C-terminal domain of lamin A was confirmed by the ability of GST-lamin A\textsubscript{389} to pull down \textit{in vitro}-translated full-length lamin A (data not shown).

To demonstrate that the lamin A–SREBP1 interaction occurs \textit{in vivo}, SREBP1\textsubscript{a} and full-length myc-tagged lamin A were co-expressed in 293T cells and an immunoprecipitation was performed using anti-myc antibody 9E10, on 293T cells expressing myc-lamin A (lane 1). SREBP1\textsubscript{a} (lane 2) or both proteins (lane 3). Expression of myc-lamin A (top panel) and SREBP1\textsubscript{a} (middle panel) was confirmed by immunoblot of whole cell supernatants. Co-precipitating SREBP1\textsubscript{a} was detected by immunoblot using an anti-SREBP1 antibody (lower panel).

\textbf{Lamin A interacts with the N-terminal transcription factor domain of SREBP1}

SREBP proteins are regulated by a novel mechanism (41) and are produced as membrane-bound precursors, composed of an N-terminal transcription factor domain and a C-terminal regulatory domain, separated by two transmembrane domains (Fig. 2). This precursor resides in the endoplasmic reticulum, where the regulatory domain interacts with SREBP-cleavage activating protein (SCAP), a protein able to sense the cholesterol content of the membrane. When the cholesterol content is lowered, the SREBP–SCAP complex is translocated to the Golgi where cleavage by two proteases releases the mature N-terminal domain, which is then able to enter the nucleus via nuclear pores and bind to enhancer regions of the appropriate genes.

To determine which SREBP1 domain is involved in binding to lamin A, we expressed GST-lamin A\textsubscript{389} in bacteria and performed pull-down assays with \textit{in vitro}-translated domains of SREBP1. The N-terminal domains of SREBP1\textsubscript{a} (residues 1–487), SREBP1\textsubscript{c} (1–463) and SREBP1\textsubscript{t} (227–487) were incubated with GST-lamin A\textsubscript{389} immobilized on glutathione–Sepharose beads. Top panel, 10% of input \textit{in vitro} translation product. Lower panel, proteins bound to GST-lamin A\textsubscript{389}.

Figure 3. \textit{In vitro} and \textit{in vivo} confirmation of the SREBP1–lamin A interaction.

(A) \textit{In vitro}-translated, \textsuperscript{35}S-labelled SREBP1\textsubscript{a} (10% of input shown in lane 1) was incubated either with GST (lane 2) or with GST-lamin A\textsubscript{389} in the absence (lane 3) or presence (lane 4) of 10 mM GMP-PNP, previously immobilized on glutathione–Sepharose beads. Following SDS–PAGE, bound proteins were detected by autoradiography. (B) Immunoprecipitations were performed using anti-myc antibody 9E10, on 293T cells expressing myc-lamin A (lane 1). SREBP1\textsubscript{a} (lane 2) or both proteins (lane 3). Expression of myc-lamin A (top panel) and SREBP1\textsubscript{a} (middle panel) was confirmed by immunoblot of whole cell supernatants. Co-precipitating SREBP1\textsubscript{a} was detected by immunoblot using an anti-SREBP1 antibody (lower panel).

Figure 4. Binding of SREBP1 domains to GST-lamin A\textsubscript{389}. \textit{In vitro}-translated, \textsuperscript{35}S-labelled full-length (FL) SREBP1\textsubscript{a}, SREBP1\textsubscript{a} (1–487), SREBP1\textsubscript{c} (1–463) and SREBP1\textsubscript{t} (227–487) were incubated with GST-lamin A\textsubscript{389} immobilized on glutathione–Sepharose beads. Top panel, 10% of input \textit{in vitro} translation product. Lower panel, proteins bound to GST-lamin A\textsubscript{389}.

diphosphate (GMP-PNP), which has been shown to dissociate importin–cargo complexes (40). As shown in Figure 3A (lane 4), SREBP1\textsubscript{a} bound to GST-lamin A\textsubscript{389} even in the presence of GMP-PNP, thus indicating that the interaction is not mediated by importins.
both isoforms of SREBP1 are capable of interaction with lamin A. Unfortunately, we were unable to express the SREBP1 C-terminal domain, possibly due to the fact that this domain is unstable and rapidly degraded; therefore, it cannot be ruled out that lamin A also interacts with this domain.

Taken together with the fact that the shortest mouse SREBP1 clone isolated in the library screen extended from amino acid 220 (equivalent to amino acid 227 of human SREBP1a), these results suggest that a binding site for lamin A lies between amino acids 227 and 487 of human SREBP1a. To confirm this, the pull-down experiment was performed with a further deletion mutant, comprising SREBP1 residues 227–487, which was successfully precipitated (Fig. 4, lane 4). Therefore, a lamin A binding site is located within residues 227–487 of SREBP1, a region that includes the bHLH-Zip motif.

Quantitative effects of disease mutations on the lamin A and SREBP1 interaction

To determine the effects of FPLD mutations on the interaction of lamin A with SREBP1, the mature, N-terminal domain of SREBP1c was expressed as a GST fusion protein and immobilized on glutathione–Sepharose beads. Pull-down experiments were performed using in vitro-translated wild-type and mutant forms of full-length lamin A. Three FPLD-specific mutants, G465D, R482W and K486N were tested, as well as an EDMD-AD (L530P) and a CMD1A (E203G) mutant. Interestingly, all of the FPLD mutations resulted in a decrease in binding of lamin A to SREBP1c (Fig. 5). The three FPLD mutants reduced binding by 25–45%, the greatest decrease being observed for G465D. These three residues may therefore sit directly in the SREBP binding site and make key interactions with the SREBP protein. Surprisingly, the most dramatic decrease in binding was observed with the EDMD-AD mutant, L530P. Here, binding was reduced by 62%. In contrast, the CMD1A mutation, E203G, appeared to have no effect on the binding of lamin A to SREBP1c. Similar results were also obtained with SREBP1a (data not shown).

DISCUSSION

Based upon the results that we have obtained here, FPLD mutations do not appear to influence either the expression or localization of lamin A/C. Equally, the EDMD-AD mutation L530P does not affect lamin A localization. In contrast, the CMD1A mutation E203G resulted in total mislocalization of lamin A to intranuclear foci. This result supports the hypothesis that CMD1A mutations, due to their clustering within the coiled-coil domain of lamin A/C, act by disrupting dimerization or polymerization of the protein. Additional studies of the effects of mutations on lamin A/C expression have recently been published by several other groups (42–44). In all cases, FPLD mutations had no effect on either lamin A/C expression or localization, although Vigouroux et al. (44) did find that FPLD mutations cause changes in nuclear morphology. In contrast, LMNA mutations causing both EDMD-AD and CMD1A were found to have varying effects on lamin A localization, with some mutants showing normal distribution whereas others displayed the punctate nuclear appearance that we observed for the E203G mutant (42,43). However, Ostlund et al. (42) did not observe the intranuclear foci for the E203G mutant, which may be due to their use of a mouse myoblast cell line, rather than human HeLa cells. Raharjo et al. (43) demonstrated that lamin C localization was affected in all the EDMD-AD and CMD1A mutants tested. The localization of other components of the nuclear lamina was also disrupted by mislocalizing lamin A/C mutants. EDMD-AD and CMD1A may therefore represent variations of a common disease phenotype (43,45), perhaps through effects on nuclear envelope structure due to mislocalization of lamin A and/or lamin C. On the other hand, FPLD evidently arises through a different effect on lamin A/C function.

To identify adipocyte-specific lamin A interacting proteins that may mediate the action of LMNA mutations in FPLD, we performed a yeast two-hybrid interaction screen of a 3T3-L1 adipocyte library using the C-terminal domain of lamin A as bait. One of the interacting proteins identified was lamin A itself, which is not unexpected, since lamin A/C polymerizes to form a filamentous network. However, this is the first demonstration of a direct interaction of the globular domain of lamin A with itself. The α-helical rod domain has previously been shown to be required for the dimerization of lamin A, whereas the N- and C-terminal domains are thought to promote lateral association to form filaments (3). Dimerization of the C-terminal domain may therefore be involved in lateral
assembly of lamin A/C protofilaments. It is of interest that only lamin A was isolated as interacting partner to the lamin A bait, whereas no lamin C clones were identified. This may reflect the preferential formation of lamin A homodimers rather than heterodimers with lamin C or, alternatively, the relative abundance of lamin A compared to lamin C mRNA in the 3T3-L1 adipocyte cell line.

The adipocyte differentiation factor, SREBP1, was also isolated in the yeast two-hybrid screen and the interaction confirmed by both in vitro GST pull-down and in vivo co-immunoprecipitation experiments. Importantly, the SREBP1 sequences required for this interaction were localized to amino acids 227–487, which lie within the N-terminal transcription factor domain of the protein. This domain enters the nucleus upon activation and cleavage of full-length SREBP1 precursor and, thus, is capable of interaction with nuclear lamin A.

The functional relevance of the lamin A-SREBP1 interaction is not yet clear; however, there are several possibilities. Since the interaction occurs between lamin A and the mature transcription factor domain of SREBP1, lamin A may facilitate the import or localization of SREBPs in the nucleus. Alternatively, lamin A may be more directly involved in the formation, stabilization or regulation of transcription complexes involving SREBP proteins. Mutations in LMNA that affect this interaction may disrupt SREBP1 function, thus interfering with the differentiation of adipocytes.

SREBP2 was also identified as a lamin A interacting protein in this study. SREBP2 is expressed at only low levels in adipose tissue, being principally involved in the regulation of cholesterol biosynthesis in the liver (36). However, SREBP1 and SREBP2 share 71% homology within the bHLH-Zip motif, a potential explanation for this result. Interestingly, serum high density lipoprotein-cholesterol levels are reduced in FPLD patients (46), suggesting that SREBP2 function may also be disrupted. Alternatively, it is possible that FPLD mutations do not affect the interaction of SREBP2, which may contact different sidechains within the C-terminal domain of lamin A/C.

To test the biological relevance of the SREBP1-lamin A interaction to disease, we examined the ability of GST-SREBP1 to pull down lamin A mutants representing the three LMNA gene disease phenotypes. FPLD mutants did exhibit reduced binding to SREBP1 by 25–45%, suggesting that a possible mechanism by which LMNA mutations lead to lipodystrophy may be due to SREBP1 dysfunction, as proposed by Hegele (47). These data also support our proposal that residues mutated in FPLD form a binding site for an interacting protein. Although lamin A binding was not completely abolished by the mutations, the reduction may be sufficient to significantly impair the function of SREBP1 in the cell.

The EDMD-AD mutation, L530P, also reduced lamin A binding to SREBP1. L530 lies within the C-terminal domain of lamin A and, although this residue is predicted to be buried within the protein (S. Shoelson, personal communication), the leucine to proline mutation could result in conformational changes that are likely to affect the surface structure of the protein, such that the binding site for SREBP1 is altered. Interestingly, varying degrees of lipodystrophy have been observed in EDMD-AD patients (F. Muntoni, personal communication). Indeed, a lamin A knockout mouse, as well as displaying the phenotype of EDMD, also lacks subcutaneous fat (25). On the other hand, the E203G mutation, which lies within the coiled-coil domain of the protein, did not appear to cause a reduction in binding of lamin A to SREBP1c. Instead, the results of our localization studies suggest that this mutation acts by disrupting dimethylation and or subsequent polymerization of lamin A due to its aggregation within the nucleoplasm. Although lipodystrophy has not yet been observed in CMD1A patients, the findings of Ostlund et al. (42) and Raharjo et al. (43) suggest that the same disease mechanisms may underlie both EDMD-AD and CMD1A. Thus, it is possible that some CMD1A mutations may also result in the development of lipodystrophy. The fact that most CMD1A mutations lie within the coiled-coil domain of lamin A/C would, however, argue against this as a general rule, since these mutations are less likely to affect the structure of the C-terminal globular domain of the protein. These issues may be resolved by the analysis of a broader spectrum of lamin A/C mutations with respect to SREBP1 binding, alongside detailed clinical assessment of patients carrying each of the mutations for evidence of lipodystrophy.

**MATERIALS AND METHODS**

**Plasmid constructs**

Sequences of primers used for PCR amplification are shown in Table 1. pGBDU-mLMNA379 was generated by PCR amplification of codons 379–664 of the mouse lamin A cDNA using primers m379-F and mLMNA-R. The PCR product was digested with EcoRI and BglII and ligated into the same sites in pGBDUc1 (32). Full-length human lamin A cDNA was obtained from IMAGE clone 897544 (48). pGBDU-hLMNA389 was generated by PCR amplification using primers h389-F and hLMNA-R. The product was digested with EcoRI and BamHI and ligated into the same sites in pGBDUc1. pGEX-hLMNA389 was generated by excision of the insert from pGBDU-hLMNA389 with EcoRI and Sall and ligation into the same sites in pGEX-4T3 (Amersham-Pharmacia). To create a myc-tagged lamin A expression vector, the insert was excised from IMAGE clone 897544 and ligated into pcIneo (Promega) via the Sall and NolI sites common to both vectors. A myc tag was engineered into the 5′ end of the lamin A cDNA by PCR, using primers hLMNAnyc-F and hLMNA1-R. The PCR product was digested with EcoRI and AccI and used to replace the 5′ end of the lamin A cDNA in pcIneo, creating pC-mycLMNA. Point mutations G465D, R482W, K486N, L530P and E203G were introduced into lamin A constructs using either QuickChange (Stratagene) or Transformer (Clontech) site-directed mutagenesis kits, according to the manufacturer’s protocol and using primers listed in Table 1.

Full-length human SREBP1a cDNA was obtained from IMAGE clone 2230916 (48). pGAD-hSREBP1a was produced by PCR amplification of the entire SREBP1a coding sequence using primers SREBP1a-F and SREBP1a-R. The product was digested using BamHI and Sall then ligated into the same sites in pGAD-GH (Clontech). pC-SREBP1a was created by digesting pGAD-SREBP1a with Sall and partially digesting with EcoRI. The 3.5 kb partial digest fragment was cloned into the same sites in pcIneo. pcDNA-SREBP1a(1–487), pcDNA-SREBP1c(1–463) and pcDNA-SREBP1(227–487) were generated by PCR amplification, using primers SREBP1N-R and either SREBP1a-F, SREBP1a-N or SREBP1c-F.
SREBP1c-F or SREBP1-227, respectively. The products were digested with BamHI and XhoI and cloned into the same sites in pcDNA3 (Invitrogen). pGEX-SREBP1a(1-487) and pGEX-SREBP1c(1-463) were produced in a similar manner, by ligating the PCR products into the BamHI and SalI sites of pGEX-4T-3 (Amersham-Pharmacia).

All constructs were verified by automated DNA sequencing on an Applied Biosystems 377 sequencer using Applied Biosystems BigDye Terminator sequencing kit (Perkin-Elmer).

**Yeast two-hybrid analysis**

Yeast strain PJ69-4a (32) was transformed using the method described by Agatep *et al.* (49). For the library screen, PJ69-4a was transformed with the bait plasmid pGBDU-mLMNA379 and maintained on SD/-URA. This strain was then transformed with 60 µg of mouse 3T3-L1 adipocyte cDNA library DNA. Transformants were plated onto SD/-URA/-LEU/-HIS supplemented with 10 mM 3-aminotriazole (3-AT). Positive clones were then plated onto SD/-URA/-LEU/-HIS/-ADE/+ 10 mM 3-AT. The library plasmid was isolated from any remaining positives by firstly performing a negative selection on SD/-LEU supplemented with 1 g/l 5-fluoro-orotic acid, to select for cells carrying library plasmid only. Plasmid DNA was then isolated using a Zymoprep kit (ZymoResearch) and inserts sequenced using oligonucleotides ADF (5’-TACCACTACAATGGGATG-3’) and T7.

**GST pull-down assays**

GST fusion proteins were expressed by transformation of the appropriate pGEX constructs into *E. coli* strain BL21 and induction with 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 1–3 h at 37°C. Proteins were purified using glutathione-Sepharose 4B (Amersham-Pharmacia) following the manufacturer’s protocol. 35S-labelled lamin A and SREBP1 proteins were produced by in vitro translation of the relevant pCIneo and pcDNA3 constructs using the TNT T7-coupled transcription/translation system (Promega), according to the manufacturer’s recommendation. Approximately 10–20 µg of GST fusion protein (immobilized on glutathione-Sepharose beads) were added to 20 µl of in vitro translation product and the final volume adjusted to 750 µl with NETN buffer (0.5% Nonidet P-40, 1 mM EDTA, 20 mM Tris–HCl pH 8, 100 mM NaCl). This mixture was incubated at 4°C for 90 min or overnight and then washed four times in NETN buffer. Proteins bound to the beads were eluted by boiling in 2× Laemmli buffer. Samples were resolved on 10% polyacrylamide gels and subjected to Coomassie staining and autoradiography. Densitometry was performed using Genesnap and Genetools (Syngene).

**Cell culture and transfections**

FPLD patient and wild-type (142BR) skin fibroblasts, HeLa and 293T cells were grown at 37°C and 5% CO2 in DMEM supplemented with 10% fetal calf serum and antibiotics. Transfections were performed using FuGene 6 reagent (Roche), according to the manufacturer’s recommendation. 293T cells were seeded onto 10 cm dishes and transacted with 2 µg each of pCI-mycLMNA and pCI-SREBP1a (or 2 µg of empty pCIneo in place of either one). HeLa cells, grown on

<table>
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<th>Primer name</th>
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Table 1. Primers used for PCR-based cloning and mutagenesis
Cells were grown to confluence on 10 cm dishes. For direct immunofluorescence, cells were lysed in buffer containing 50 mM Tris–HCl pH 7.4, 100 mM NaCl, 10 mM MgCl₂, 1% Triton X-100, 10 µg/ml DNase I, 10 µg/ml RNase A and 1 mM PMSF. Lysates were incubated on ice for 30 min, passed through a 27 gauge needle and centrifuged to obtain soluble and insoluble fractions. Samples were boiled in 1× Laemmli buffer and resolved on 7.5% polyacrylamide gels.

Proteins were transferred onto nitrocellulose for 1 h at 70 mA using a Hoefer semi-dry blotting apparatus. Primary antibodies were diluted in blocking buffer containing 50 mM Tris–HCl pH 7.4, 100 mM NaCl, 10 mM MgCl₂, 1% Triton X-100, 10 µg/ml DNase I, 10 µg/ml RNase A and 1 mM PMSF. Lysates were incubated on ice for 30 min, passed through a 27 gauge needle and centrifuged to obtain soluble and insoluble fractions. Samples were boiled in 1× Laemmli buffer and resolved on 7.5% polyacrylamide gels.

Electron microscopy was performed using a Zeiss Axioskop 2 microscope.

Immunoprecipitations

Protein extracts were obtained by incubation of confluent cell monolayers on ice with 600 µl of lysis buffer containing 50 mM HEPES, 1% Triton X-100, 150 mM NaCl, 1 mM PMSF and Complete™ protease inhibitors (Roche). Cell debris was removed by centrifugation and a 50 µl sample removed for direct analysis by immunoblotting. The remaining supernatant was pre-cleared by incubation at 4°C with protein A–Sepharose beads (Amersham-Pharamacia), followed by centrifugation. The supernatant was incubated at 4°C for 2 h with 2 µg of 9E10 anti-myc antibody (Zymed). Protein A–Sepharose beads were then added and the incubation continued for a further 1 h. The beads were washed with lysis buffer and solubilized in 2× Laemmli buffer. Samples were boiled for 5 min prior to separation on 7.5% polyacrylamide gels and immunoblotting.

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