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Subcellular localization of SREBP1 depends on its interaction with the C-terminal region of wild-type and disease related A-type lamins

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Abstract

Lamins A and C are nuclear intermediate filament proteins expressed in most differentiated somatic cells. Previous data suggested that prelamin A, the lamin A precursor, accumulates in some lipodystrophy syndromes caused by mutations in the lamin A/C gene, and binds and inactivates the sterol regulatory element binding protein 1 (SREBP1). Here we show that, *in vitro*, the tail regions of prelamin A, lamin A and lamin C bind a polypeptide of SREBP1. Such interactions also occur in HeLa cells, since expression of lamin tail regions impedes nucleolar accumulation of the SREBP1 polypeptide fused to a nucleolar localization signal sequence. In addition, the tail regions of A-type lamin variants that occur in Dunnigan-type familial partial lipodystrophy (R482W) and Hutchison Gilford progeria syndrome (Δ 607–656) bind to the SREBP1 polypeptide *in vitro*, and the corresponding FLAG-tagged full-length lamin variants co-immunoprecipitate the SREBP1 polypeptide in cells. Overexpression of wild-type A-type lamins and variants favors SREBP1 polypeptide localization at the intranuclear periphery, suggesting its sequestration. Our data support the hypothesis that variation of A-type lamin protein level and

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⁺Deceased

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spatial organization, in particular due to disease-linked mutations, influences the sequestration of SREBP1 at the nuclear envelope and thus contributes to the regulation of SREBP1 function.

Keywords

lamin; SREBP1; nuclear envelope; lipodystrophy; progeria

Introduction

The nuclear lamina is an intermediate filament meshwork composed of A-type and B-type lamins. In mammals, the B-type lamins are expressed in most or all nucleated cells and are encoded by the *LMNB1* and *LMNB2* genes. In contrast, the A-type lamins are mainly expressed in differentiated somatic cells and arise from alternative RNA splicing of the *LMNA* gene. This splicing gives rise to prelamin A (the precursor of lamin A), lamin C, lamin A Δ 10 and the germ cell-specific lamin C2 [1–3]. Prelamin A and lamin C differ only in their C-terminal regions: lamin C has 6 specific C-terminal residues, while prelamin A exhibits 98 unique residues [1]. Unlike lamin C, prelamin A undergoes a multistep posttranslational processing at its C-terminal CaaX motif involving 3 enzymes. Initially a farnesyl moiety is added to the cysteine by a protein farnesyltransferase. This allows cleavage of the last 3 amino acids (-aaX) by ZmpSte24. Then the C-terminal cysteine is carboxymethylated by an isoprenylcysteine carboxymethyl transferase to generate a farnesylated/carboxymethylated prelamin A. Finally, within approximately 90 min of synthesis [4], ZmpSte24 cleaves the prelamin A 15 C-terminal amino acids to generate a mature lamin A [5]. Research on prelamin A and lamin A functional roles has been stimulated by the discovery of diseases caused by mutations in *LMNA*, often called laminopathies associated with mutations in *LMNA*, which include skeletal and/or cardiac myopathies, lipodystrophy, mandibuloacral dysplasia, peripheral neuropathy and premature ageing [6,7]. However, the biological significance of the prelamin A maturation process is still unclear.

Lamins have a central α -helical rod domain flanked by a small N-terminal head and a large C-terminal tail. The tail regions of lamins contain a globular Ig-like domain [8,9] and a large sequence predicted as unstructured. This sequence is modified during the maturation process. Amino acid substitutions and small deletions occur throughout the tail region in several diseases, including Dunnigan-type familial partial lipodystrophy (FPLD), which affects adipose tissue, and Hutchinson-Gilford progeria syndrome (HGPS), which has features of premature aging. These two diseases have very different phenotypes but share as a common feature loss of subcutaneous fat [10]. Defects in prelamin A processing have been observed in both of these diseases, as well as in restrictive dermopathy [5, 11–13]. In HGPS and restrictive dermopathy, the prelamin A processing defect mechanisms are clear: specific mutations in *LMNA* that cause HGPS lead to expression of a truncated prelamin A variant called progerin that lacks a cleavage site critical for prelamin A processing [14,15]; heterozygous loss of function mutations in *ZMPSTE24* encoding the prelamin A processing enzyme occur in subjects with the neonatal lethal progeroid syndrome called restrictive dermopathy [5]. However, in the case of FPLD, the mechanisms leading to accumulation of prelamin A are still a matter of debate. Indeed, it has been reported that *LMNA* mutations causing FPLD most frequently lead to amino acid substitutions changing the charge of the Ig fold surface in lamins A and C [8,9]. These mutations could hinder the binding of prelamin A to a yet unidentified partner regulating its processing.

Lamins bind transcription factors [16]. Searches for lamin binding partners in adipocytes identified the sterol response element binding protein 1 [17], a transcription factor that

regulates the expression of genes involved in cholesterol biosynthesis and adipogenic differentiation [18]. SREBP1 is a member of the basic-helix-loop-helix (bHLH)-leucine zipper family of transcription factors synthesized as an inactive precursor embedded in the endoplasmic reticulum membrane [19]. Depletion of cholesterol leads to intra-membrane proteolysis, releasing the active portion of SREBP1 containing the basic DNA-binding region from the endoplasmic reticulum membrane. After translocation to the nucleus and binding to its specific DNA sequences, SREBP1 dimers induce the expression of target genes involved in adipogenesis and membrane biogenesis [20]. It has been reported that *in vitro* region 227 to 487 of SREBP1 encompassing its DNA binding domain interacts with region 389 to 664 in the tail of prelamin A [17]. Pools of SREBP1 have also been observed at the nuclear periphery of cells from human subjects with FPLD and two other diseases caused by *LMNA* mutations that have lipodystrophy as a feature, mandibuloacral dysplasia (MAD) and atypical Werner's syndrome [12]. In these cells, SREBP1 co-immunoprecipitates with prelamin A but not with lamin A or lamin C [12]. In mouse pre-adipocyte cell lines overexpressing prelamin A, expression of PPAR γ that depends on SREBP1 is reduced and differentiation is impaired [12]. Based on these data, Capanni et al. [12] have proposed that the interaction between SREBP1 and prelamin A downregulates SREBP1 activity. It was later suggested that SREBP1 can also interact in the nucleoplasm with unfarnesylated prelamin A [21,22], which accumulates in cells treated with drugs that block protein prenylation.

Here we focus on the *in vitro* and *in vivo* recognition of the SREBP1 polypeptide 227 to 487 by diverse A-type lamin isoforms and disease related variants. We first address the question of whether the interaction between the SREBP1 polypeptide and A-type lamins is restricted to prelamin A *in vitro* and in cells. We further investigate the impact of overexpressing the tail region of lamins as well as the full-length wild-type and disease related mutant lamins on the localization of the SREBP1 polypeptide in cells. We then discuss how lamin sequence variations and/or protein levels can justify an eventual change in SREBP1 sequestration efficiency.

Materials and Methods

Plasmid constructs

Constructs encoding glutathione-S-transferase (GST) fusions of the C-terminal fragments of lamins (amino acids 389-664 of prelamin A, 389-646 of lamin A, 389-572 of lamin C and 391-586 of lamin B1) as well as the Ig fold domain common to lamin A and lamin C (amino acids 411-553) were generated by PCR using as templates the corresponding pSVK3-lamin vectors described in Favreau et al. [23]. Amplified DNA was ligated into the BamHI and EcoRI sites of pGEX-4T. Constructs encoding the GST-C-terminal fragments of R482W prelamin A and lamin A mutants were generated using as a template the pSVK3-FLAG-prelamin A R482W and ligating the amplified DNA into the BamHI and EcoRI sites of pGEX-2T. A construct encoding a GST fusion of the C-terminal fragment of progerin (amino acids 389-664 with a deletion of residues 607 to 656 of prelamin A) was generated using as template the plasmid pEGFP-progerin [24], followed by ligation of the amplified DNA into the BamHI and EcoRI sites of pGEX-2T. The GST fusion protein containing the sequence deleted in the progerin (amino acids 607-656) was obtained by ligation of the corresponding DNA amplified using as template the plasmid pSVK3-FLAG-prelamin A WT, into the BamHI and EcoRI sites of pGEX-2T.

A construct encoding a His fusion protein of SREBP1 from amino acids 227 to 487 (numbering according to the human SREBP1a sequence (UniProt ID : P36956)) was generated by PCR amplification of the relevant region of cDNA using as template pBluescriptR-SREBP1 (imaGenes), followed by ligation of the amplified DNA into the

NcoI and BamHI sites of pETM10. Hemagglutinin (HA) and green fluorescent protein (GFP) fusions of SREBP1 were generated by PCR amplification of the DNA regions coding for residues 227-487 of SREBP1 using as template pBluescriptR-SREBP1 (imaGenes). Amplified DNA was ligated into either the EcoRI and XbaI sites of pCDNA3-3HA (generous gift from Dr. S. Pichon) or the SalI and BamHI sites of pEGFP-C1 (Clontech). A SREBP1 construct containing a nucleolar localization sequence (Nols) was generated by inserting Nols into a plasmid encoding the GFP-SREBP1 fusion. Excision of the Nols sequence from the plasmid pEGFP-Nols-Nop56 [25] and subsequent ligation into the pEGFP-SREBP1 fusion construct were performed using the XhoI and SalI sites.

Constructs encoding a GFP fusion of lamin B1 and FLAG fusions of wild-type prelamin A, prelamin A R482W and wild-type lamin C have been previously described [23,26]. pSVK3 constructs encoding FLAG-tagged prelamin A L647R, FLAG-tagged lamin A and FLAG-tagged lamin B1 were generated following previously described procedures [27]. pSVK3 construct encoding FLAG-tagged progerin was generated by first digesting with the restriction enzymes Age I and XhoI both the pEGFP-progerin vector [24] and the pSVK3-FLAG-prelamin A vector, and then by ligating the purified DNAs corresponding on one side to the aa region [481-*del607-656-664*] and on the other side to the pSVK3 vector encoding FLAG-tagged prelamin A 1-481. Constructs encoding FLAG fusions of mini lamin A (amino acids 1-431), the C-terminal of prelamin A (amino acids 389-664) and the C-terminal of lamin A (amino acids 389-646) were generated by PCR amplification of the relevant regions of cDNA using as template pSVK3-prelamin A, followed by ligation of the amplified DNA into the BamHI and EcoRI sites of pCMV-Tag 2A (Stratagene). The construct encoding a FLAG fusion of the C-terminal of lamin C (amino acids 389-572) was generated by PCR amplification of the relevant region of cDNA using as template pEGFP-lamin C (generous gift of Dr. J. Broers), followed by ligation of the amplified DNA into the BamHI and EcoRI sites of pCMV-Tag 2A (Stratagene).

Production of SREBP1 fragments

The N-terminal His-tagged polypeptide of SREBP1 from amino acid 227 to 487 was produced in *Escherichia coli* BL21(DE3)star. Expression was induced by 0.5 mM isopropyl-D-1-thiogalactopyranoside when OD_{600nm} reached 1.0 and the culture was then incubated overnight at 20°C. Cells were disrupted by sonication in 50 mM phosphate buffer, pH 8.0, containing 150 mM NaCl, 20 mM imidazole, 5 mM β-mercaptoethanol and 1 mM phenylmethanesulfonylfluoride. Then, the lysate was loaded onto a His Trap column 5 ml (GE Healthcare) and His-tagged proteins were eluted with an imidazole gradient from 20 mM to 1M. After buffer exchanging using a PD-10 desalting column (GE Healthcare), the proteins were loaded onto a HiTrap heparin column 5 ml (GE Healthcare) and eluted by a NaCl gradient from 50 mM to 2 M in 20 mM phosphate, pH 7.0, containing 5 mM β-mercaptoethanol. Quantification of proteins was performed using the Bradford assay [28].

Production of lamin A and lamin C fragments

The fragments of the C-terminal regions of lamins A and C were produced in *Escherichia coli* BL21 as GST-fusion proteins. Expression was induced by 1 mM isopropyl-D-1-thiogalactopyranoside when OD_{600nm} reached 1.0. Then, the culture was incubated at 37°C for 2.5 h for all lamins except for prelamin A and progerin for which the incubation was reduced to 45 min in order to minimize endoproteolytic degradations that occur preferentially on those isoforms. Purification of the GST-lamin fusion proteins was performed according to the manufacturer's instructions, using glutathione-Sepharose 4B beads (GE Healthcare, Amersham). Cells were disrupted by sonication in phosphate-buffered saline containing 1 mM phenylmethanesulfonylfluoride and 1% Triton X-100. Briefly, the bacterial lysate was incubated with beads for 1.5 h at 4°C. After washes in 50

mM Tris, pH 7.5, containing 5 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF) in order to remove traces of contaminating bacterial DNA, recombinant proteins were treated with DNase (10 µg/ml in 50mM Tris, pH 7.5, 5 mM MgCl₂, 10% glycerol, 5 mM AEBSF) for 30 min at room temperature. After 3 washes with 50 mM Tris, pH8.0, 1 mM ethylenediaminetetraacetic acid, 1% Triton X-100, 1 mM AEBSF that progressively contained decreasing concentrations of NaCl (600 mM, 300 mM and 150 mM), GST chimeric proteins were eluted with 10 mM reduced glutathione diluted in 50 mM Tris, pH 8.0, after incubation at 4°C for 90 min. Aliquots of GST chimeric proteins were kept frozen at -80°C. Alternatively, GST was removed by treating fusion proteins immobilized on beads with thrombin protease. Lamin fragments were recovered in the soluble fraction and protein concentration was immediately determined by measuring optical absorbance at 280 nm.

***In vitro* pull-down assays**

Glutathione-Sepharose 4B beads (20 µl) were first saturated with GST-lamin fusion proteins or GST-only (500 pmoles) in 150 µl of Tris-DTT buffer (50 mM Tris, pH 8.0, 1 mM DTT, 0.5% Triton X-100, 1 mM AEBSF) and 1M NaCl at 4°C for 2 h. After 2 washes, the His-tagged SREBP1 polypeptide was added (1,000 pmoles in 150µl of Tris-DTT buffer and 150 mM NaCl) to the saturated beads and incubation was carried out at 4°C for 1.5 h. Beads were then washed 4 times with Tris-DTT buffer and 150 mM NaCl. Lamin-SREBP1 protein complexes bound to glutathione beads were released by adding 40 µl of 2X Laemmli buffer [29], boiled for 5 min, separated on 10% SDS-polyacrylamide gels, transferred to nitrocellulose membranes (Macherey-Nagel) using the semi-dry BioRad system and analyzed by western blotting. His-SREBP1 proteins were detected using an anti SREBP1 antibody. Detection of GST-tagged purified proteins was performed directly on nitrocellulose membranes by red Ponceau staining, while the presence of the GST tag was confirmed using an anti GST antibody (data not shown).

Western blot

Proteins were first separated on SDS-polyacrylamide gels and transferred to nitrocellulose membranes. Then, nitrocellulose membranes were blocked for 1.5 h in TBS buffer (10 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween-20) containing 5% dry milk, incubated with the appropriate primary antibodies for 1 h in TBS buffer containing 1% milk, washed 4 times for 5 min each and then incubated with HRP-conjugated secondary antibodies. After 4 washes with TBS buffer, enhanced chemiluminescence (ECL, West pico system; Pierce) was used for detection of signals and images were captured with Fuji LAS-4000. When necessary, ECL signals were quantified by densitometry using ImageJ (NIH). SREBP1 ECL signals obtained for pull-down assays were normalized with respect to the amount (moles) of recombinant GST tagged proteins (GST^o or GST-cter-lamins) effectively detected on the nitrocellulose membrane used for the immunoblot. Red ponceau staining of GST-cter prelamins A samples revealed one native band (~59kDa) and two major degradation products (~50kDa). We only considered the native form and one of the degradation products that still contains the SREBP1 binding site as assessed by blot overlay (data not shown) to estimate the amount of GST-cter prelamins A. Primary antibodies used for western blots were rabbit anti SREBP1 (K10) from Santa Cruz Biotechnology (dilution 1:300 or 1:600 for analysis of proteins related to either co-immunoprecipitation or pull-down experiments, respectively), mouse anti FLAG (M2) from Sigma (dilution 1:600), rabbit anti FLAG from Sigma (dilution 1:350), goat anti prelamins A (C-20) from Santa Cruz Biotechnology (dilution 1:300), and mouse anti GST (B-14) from Santa Cruz Biotechnology (dilution 1:3000).

Isothermal Titration Calorimetry (ITC)

To measure the binding of lamin fragments to SREBP1 fragments, ITC was performed using a VP-ITC Microcalorimeter (MicroCal). Proteins were dialyzed against 50 mM Tris, pH 7.5, and 150 mM NaCl before measurements. Experiments were performed at 25°C. In a typical experiment, the cell contained 2 μ M of SREBP1 polypeptide and the syringe contained 60 μ M of lamin polypeptide. Data were analyzed using MicroCal Origin software and fitting to a single site model.

Cell culture and transfection

HeLa cells were obtained from American Type Culture Collection and grown in Minimum Essential Medium containing glutamax (Gibco), 1% non-essential amino acids and 10% fetal bovine serum. For cell transfection, FuGENE 6 or XtremeGENE 9 were used according to the manufacturer's instructions (Roche). Cells were analyzed 24 h after transfection.

Immunofluorescence microscopy

Cells were fixed with 3% paraformaldehyde solution in PBS for 12 min at room temperature and further processed for immunofluorescence microscopy. In some instances, cells were submitted to pre-extraction with non-ionic detergents and salt before fixation, a treatment that mainly removes soluble and cytoskeletal proteins but not lamin and associated proteins [30]. Briefly, cells were first incubated in 100 mM NaCl, 300 mM sucrose, 10 mM PIPES, pH 6.8, 3 mM MgCl₂ and 0.5% Triton X-100 for 10 min at 4°C and then in 250 mM ammonium sulfate, 300 mM sucrose, 10 mM PIPES, pH 6.8, 3 mM MgCl₂ and 0.1% Triton X-100 for 5 min at 4°C. Primary antibodies were rabbit anti SREBP1 (K10) from Santa Cruz Biotechnology (dilution 1:50), mouse anti Flag (M2) from Sigma (dilution 1:300), rabbit anti lamin A/C generated against the NLS region described in Cance et al. [31] (dilution 1/400), goat anti prelamin A (C-20) from Santa Cruz Biotechnology (dilution 1:100), mouse anti HA (HA-7) from Sigma (dilution 1:150) or an autoimmune human serum directed against fibrillarin (dilution 1:3000; [32]). AffiniPure secondary antibodies (goat anti mouse, goat anti rabbit, donkey anti mouse, donkey anti goat, donkey anti rabbit, donkey anti human) conjugated to fluorophores CyTM2, CyTM3 or DyLightTM594 were purchased from Jackson ImmunoResearch.

Immunoprecipitation

HeLa cells grown in 100 mm cell culture plates were used 24 h after their transfection with the plasmids of interest. Cell lysates were prepared as described previously [12], with minor modifications. In brief, cells were lysed in buffer A (50 mM Hepes, pH 7.4, 100 mM NaCl, 2 mM MgCl₂, 1 mM EDTA, 10 % glycerol, 1% NP40) supplemented with a cocktail of protease inhibitors (Thermo Scientific), for 30 min at 4°C. After sonication, cellular debris were removed by centrifugation at 10,000 g for 10 min at 4°C. Lysates were then precleared by incubation with protein A/G beads (Santa Cruz Biotechnology, Inc) for 1.5 h at 4°C, and centrifuged at 12,000 g for 1 min at 4°C. Immunoprecipitation was then performed by incubating the "precleared" supernatant with mouse anti FLAG antibodies for 1h at 4°C, followed by addition of protein A/G agarose beads, and a further incubation of the samples for 15 h at 4°C. Control immunoprecipitation was performed with mouse anti GFP antibodies. After 4 washes in buffer B (50 mM Hepes, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.1% NP40), immunoprecipitated complexes were resuspended in 40 μ l of Laemmli buffer, and then analyzed by SDS-PAGE and western blotting. HA-SREBP1 and FLAG-lamins were revealed using rabbit antibodies directed against SREBP1 and FLAG, respectively.

Results

In vitro binding of SREBP1 to the tail region of lamin A and lamin C

We first asked whether an interaction occurs between SREBP1 and lamin A, lamin C and lamin B1 tails. The region from amino acid 227 to 487 of SREBP1a that we will call SREBP1(227-487) is sufficient to interact with the prelamin A tail [17]. Thus, we performed pull-down assays using a His-tagged fragment of SREBP1(227-487) and GST-tagged tails of either lamin A (amino acid 389-646) or lamin C (amino acid 389-572) (Fig. 1A-C). In these experiments, lamin A and lamin C tails bound SREBP1(227-487) (Fig. 1D-E). In contrast, the lamin B1 tail poorly bound SREBP1(227-487) (~4 times less than the tails of lamins A and C) (Fig. 1D-E). All lamin tails contain an Ig fold domain as well as unstructured regions. We found that the region from amino acid 411 to 553, essentially corresponding to the Ig fold common to lamins A and C, also poorly bound SREBP1(227-487) (~5 times less than the lamin C tail) (Fig. 1D-E). We then characterized by ITC the thermodynamics and stoichiometry of SREBP1 binding to lamins. We used the lamin C tail because it was the strongest SREBP1 binder in the pull-down experiments and because it was the least susceptible to degradation after removal of the GST moiety by thrombin cleavage (data not shown). In this experiment, SREBP1(227-487) bound the C-terminal region of lamin C with a dissociation constant of 63 ± 19 nM ($n=3$), a stoichiometry of 1:1, a favorable enthalpy ΔH of -214 ± 31 kcal mol⁻¹ and an unfavorable entropic contribution to the binding energy $T\Delta S$ of -204 ± 32 kcal mol⁻¹, T being the temperature of the experiment (Fig. 1F). Consistent with the GST pull-down data, ITC revealed no binding between SREBP1(227-487) and the lamin A/C Ig fold (data not shown). Thus, our data showed that the lamin A and C tails interact with the SREBP1 polypeptide and that the lamin Ig fold domain is not sufficient to bind this SREBP1 fragment, even if one can not exclude that it contributes to SREBP1 recognition.

Subcellular localization of the lamin-binding domain of SREBP1 depends on the expression of A-type lamin tails

We next asked if the interaction observed *in vitro* between SREBP1(227-487) and the A-type lamin tails occurs in cells. We performed transfection experiments to express a GFP fusion of the SREBP1 polypeptide and FLAG-tagged lamin A or lamin C tails in HeLa cells. Immunofluorescence microscopy of fixed cells revealed that both proteins accumulated in the nucleoplasm (Fig. 2A). Then we designed a cellular assay of "partners co-targeting," where SREBP1(227-487) was forced to (de)localize into a specific subregion of the nucleus, namely the nucleolus. Therefore, we generated a chimeric protein comprising SREBP1(227-487) fused to a previously defined nucleolar localization signal (Nols) sequence [25]. We transfected cells with plasmids encoding GFP fusions of the SREBP1 polypeptide with or without the Nols sequence. The GFP-SREBP1 polypeptide was located in the nucleoplasm and excluded from the nucleoli in all cells whereas the GFP-Nols-SREBP1 polypeptide fusion accumulated in nucleoli in most cells (Fig. 2A). We also co-transfected cells with plasmids that encode the GFP-Nols-SREBP1 polypeptide and either FLAG-tagged lamin A tail or FLAG-tagged lamin C tail. Expression of FLAG-tagged lamin A and C tails reduced the percentage of cells in which the GFP-Nols-SREBP1 polypeptide fusion accumulated in nucleoli (Fig. 2B, arrows) from ~69 % to ~47 and 41 %, respectively (Figure 2D). As GFP fused to a Nols sequence remained in the nucleoli of virtually all cells when FLAG-tagged lamin C tail was co-expressed, we excluded the possibility that these redistributions were due to lamin binding to the Nols sequence (Fig. 2C-D). Our results suggest that the mature A-type lamin tails interact with SREBP1(227-487) in HeLa cells.

***In vitro* binding of prelamin A and lamin A tail variants in FPLD and HGPS to SREBP1**

We asked if unprocessed prelamin A, lamin A variant R482W (FPLD) modified in its Ig fold domain, as well as prelamin A Δ 607-656, also named progerin (HGPS) would bind to SREBP1(227-487) (Fig. 3A). We tested by pull-down assays the binding of GST fusions of prelamin A or lamin A tails with or without the amino acid alterations to His-tagged SREBP1(227-487) (Fig. 3A-B). We found that all these lamin variants bound the SREBP1 polypeptide with similar avidities, whereas the GST-tagged cter-prelamin A region 607-656 that is deleted in progerin did not bind, revealing even weaker SREBP1 signals than did the control GST protein (Fig. 3B). As both wild-type prelamin A and lamin A tails recognize SREBP1(227-487), the extreme C-terminal residues specific to prelamin A, from amino acid 647 to 664, are not essential for binding to SREBP1. Moreover, the tail region of progerin binds to the SREBP1 polypeptide as wild-type prelamin A and lamin A tails, whereas the region deleted in progerin does not bind by itself, showing that amino acids 607 to 656 are not essential for SREBP1 recognition.

Wild-type lamin A and variants in FPLD and HGPS form complexes with the SREBP1 polypeptide in cells

To ascertain whether SREBP1(227-487) would effectively interact with full-length A-type lamins in cells, we performed co-immunoprecipitation experiments. Cells were transfected with the plasmid coding for HA-SREBP1(227-487) alone or together with plasmids coding for either FLAG-prelamin A wild-type, FLAG-lamin A, FLAG-prelamin A R482W, Flag-prelamin A L647R (this mutation prevents removal of the farnesylated lamin C-terminus) or FLAG-progerin. Analysis of the soluble extracts (Input) by immunoblotting revealed that FLAG-prelamin A either wild-type (Fig. 4; lanes 2, 10 and 18) or R482W variant (Fig. 4; lane 11) migrate in the polyacrylamide gels as mature lamin A, revealing their efficient processing in our experimental conditions. In contrast and as expected, while the variant prelamin A L647R migrated slower in the polyacrylamide gels due to its incapacity to be processed (Fig. 4; lane 16), the progerin variant migrated faster due to the partial deletion in its C-terminal region (Fig. 4; lane 17). Immunoprecipitation assays were performed with FLAG antibodies (Fig. 4; lanes 4, 6, 9, 13, 15, 21, 23, 25 and 26) or control antibodies against GFP (Fig. 4; lanes 3, 5, 8, 12, 14, 20, 22, 24). In the absence of FLAG-tagged lamins, the SREBP1 polypeptide was not precipitated with either GFP or FLAG antibodies (Fig. 4; lanes 3 and 4). In contrast, in the presence of FLAG-tagged lamins, the immunoprecipitations performed with anti FLAG antibodies revealed an enrichment of both lamins and SREBP1. The control immunoprecipitations revealed the absence of lamins and no enrichment in SREBP1. Therefore, overexpressed full-length prelamin A (L647R) and lamin A (expressed directly as a mature protein or generated through prelamin A processing) as well as lamin variants (lamin A R482W, progerin) were all able to form complexes with the SREBP1 polypeptide in HeLa cells.

Nuclear sub-localization of SREBP1(227-487) is influenced by the overexpression of full-length wild-type and pathological lamin A variants

Having shown that SREBP1(227-487) associates with full-length A-type lamins in cells, we next asked whether this association would have an impact on the nuclear sub-localization of the SREBP1 polypeptide. We had previously observed that the GFP-tagged SREBP1(227-487) is homogeneously distributed in the nucleoplasm of HeLa cells (see Fig. 2A). When expressed as a chimeric protein fused to the small tag HA, SREBP1(227-487) is again nucleoplasmic in most cells (Fig. 5A, upper row). However, it is observed at the nuclear periphery in 18–36% of the cells (Fig. 5A, middle row). In addition, in 4–6% of the cells, it has an inhomogeneous nucleoplasmic distribution that we refer as aggregates (Fig. 5A, lower row). Such aggregates may represent higher-order oligomers due to SREBP1 high expression levels. The peripheral SREBP1 polypeptide is adjacent to the nuclear lamina

(Fig. 5B, upper row) and, as expected for lamins and lamin-binding proteins, is resistant to extraction by non-ionic detergents and salt (successively 150 mM NaCl and 250 mM ammonium sulfate) before fixation of the cells (Fig. 5B, lower row). Such SREBP1 polypeptide distribution is reminiscent of the so-called “nuclear envelope sequestration” of a pool of endogenous SREBP1 observed in human fibroblasts from patients with lipodystrophy linked MAD (lamin A/C R527H), FPLD (lamin A/C R482L) and atypical Werner syndrome (lamin A/C S143F) [12].

We tested the impact of co-expressing the SREBP1 polypeptide HA-SREBP1(227-487) with full-length wild-type prelamin A and lamin A, the prelamin A variants R482W (FPLD) and L647R, progerin, lamin C and lamin B1. A-type lamins were expressed fused to FLAG, and lamin B1 was expressed fused to either FLAG or GFP. In control experiments, cells were co-transfected with a vector encoding SREBP1 together with a pSVK3 empty vector, or a pCMVtag vector encoding FLAG-tagged lamin A from amino acid 1 to 431, which lacks the C-terminal tail region. Initially, we observed that in mono-transfected cells tagged lamins including wild-type prelamin A, or R482W, lamin A, prelamin A L647R, progerin and lamin B1 were correctly targeted to the nuclear periphery (Fig. 6A). In contrast, we observed that the lamin A variant with the tail deletion (Flag-lamin A 1-431) accumulated within intranucleoplasmic rings in 20% of the cells (Fig. 6A). In co-transfected cells (Fig. 6B-H), three patterns of distribution were observed for the SREBP1 polypeptide as previously shown in Fig. 5. The frequency of these phenotypes varied depending on the co-expressed lamin (Fig. 6I-J). In a first set of experiments, we observed that the SREBP1 polypeptide is significantly more frequently enriched at the nuclear periphery when co-expressed with FLAG-tagged prelamin A wild-type or mutant R482W (47%–55% of the cells) than when co-expressed with either no lamin, FLAG-tagged lamin A 1-431 or GFP-lamin B1 (15%–23% of the cells) (Fig. 6I). In a second set of experiments, we observed a similar increased enrichment of SREBP1 at the nuclear periphery in the presence of either FLAG-tagged wild-type prelamin A, lamin A, uncleavable prelamin A L647R and progerin (~70–73% of the cells). The distribution occurred in only 32–36% of the cells in the absence of FLAG-tagged lamins or in the presence of FLAG-lamin B1 (Fig. 6J). In addition, we noticed that the SREBP1 aggregates were able to recruit not only wild-type lamin A (expressed directly as a mature protein or generated through prelamin A processing), but also the R482W variant lamin A, the uncleavable prelamin A L647R and progerin (Fig. 6B-C and F-H). These SREBP1 aggregates may reflect non-functional pools of SREBP1 (unable to bind their DNA sequences) that contribute to sequester partners. Inversely, the nucleoplasmic rings constituted by the truncated lamin lacking the C-terminal region were not able to recruit the SREBP1 polypeptide.

We confirmed prelamin A processing in these experiments by performing an immunoblot analysis of whole cell extracts with prelamin A specific antibodies. We observed that prelamin A is detected at high levels in cells expressing the uncleavable prelamin A (L647R; Fig. 7A lane 2 and see also Fig. 4) but is not detected in cells expressing wild-type prelamin A (Fig. 7A, lane 3). In agreement with our biochemical analysis, *in situ* detection of prelamin A using a specific antibody revealed a very strong signal at the nuclear periphery of all cells expressing prelamin A L647R (Fig. 7B) but a faint signal in cells expressing wild-type prelamin A or lamin A (Fig. 7B). Thus, although wild-type prelamin A is processed to lamin A but prelamin A L647R is not, their overexpression induces an enrichment of the SREBP1 polypeptide at the intranuclear periphery, which is apparently similar to that generated by overexpression of lamin A (Fig. 6J). We conclude that the occurrence of this SREBP1 distribution pattern in HeLa cells is independent of the ultimate degree of processing of ectopic prelamin A into lamin A.

Discussion

SREBP1 recognizes a region of A-type lamins common to prelamin A, lamin A and lamin C

We have shown that a subdomain of SREBP1 from amino acid 227 to 487 recognizes the C-terminal region of A-type lamins but poorly binds to lamin B1 *in vitro*. In cells, overexpression of prelamin A but not lamin B1 leads to an accumulation of this SREBP1 polypeptide at the intranuclear periphery. Several groups have shown that A-type and B-type lamins form distinct but connected networks [24,33–35]. These different subdomains of the lamina have specific roles in nuclear structure, anchorage of partners, binding to chromatin, chromatin organization and gene regulation [36,37]. Our findings complement these previous studies by illustrating the specificity of functions played by A-type and B-type lamins.

We have shown that the region from amino acid 389 to 572 of lamin C is sufficient to recognize SREBP1. Interaction of the lamin C tail with SREBP1 occurs with an affinity of ~60 nM which is approximately 10 times lower than the affinity of SREBP1 for its target DNA sequences [38]. Nevertheless, it is in the same range as the affinity reported between transcription factors such as fos and jun [39]. Region 389 to 664 of prelamin A, which is 100% identical to lamin C between amino acids 389 to 566, also binds SREBP1. In this case, several arguments suggest that most of the prelamin A specific region is not involved in SREBP1 binding. First, lamin A (ending at residue 646) and progerin (deleted from residues 607 to 656) also recognizes SREBP1. Second, lamin A region 607 to 656 does not bind SREBP1. Thus, lamin A/C region 389 to 566 is probably sufficient to interact with SREBP1. The inability of the lamin Ig fold domain to bind SREBP1 indicates that the lamin sequences located upstream of amino acid 411 and/or downstream from amino acid 553 participate to SREBP1 binding. These regions are predicted to be unstructured; however, we speculate that they fold upon binding to SREBP1. The Ig fold domain could also contribute, at least indirectly, to the binding, as already suggested by Shackleton and co-workers [17]. A significant decrease in SREBP1 binding avidity was previously reported for lamin A variant R482W in comparison to wild-type lamin A [17]. This discrepancy likely relies on the use of different sources of recombinant proteins, i.e full-length lamins and mature SREBP1c in the previous study [17] instead of C-terminal regions of lamins and a subregion of SREBP1 (aa 227–487 that are conserved between SREBP1a and c) in our study. In particular, changes in *in vitro* dimerisation/oligomerization properties of full-length R482W lamins versus wild-type lamins may contribute to observed changes in SREBP1 binding. However, we did not observe any impact of the R482W mutation on the localization of SREBP1 in cells.

The SREBP1 binding sites of prelamin A and lamin A/C nucleoplasmic polymers are accessible in cells

We have shown that overexpression of lamin A/C tails in HeLa cells significantly mislocalized the lamin-binding polypeptide of SREBP1 tagged with a nucleolar localization sequence from nucleoli to the nucleoplasm. The presence of SREBP1 partners such as endogenous lamins outside the nucleoli could explain why GFP-Nols-SREBP1 is observed outside of nucleoli in 31% of the cells in the absence of lamin overexpression. However, this increases to 59% and 53% after overexpression of FLAG-tagged lamin C and lamin A tails, respectively. Thus, within the A-type lamin tails that are devoid of the coil structures required for lamin oligomerization, the SREBP1 binding site is accessible. In addition, overexpressed full-length unprocessed prelamin A L647R, lamin A generated through prelamin A processing and lamin A expressed directly as a mature protein could be partly recruited within nucleoplasmic “aggregates” of the SREBP1 polypeptide. While it is known that A-type lamins polymerize both at the nuclear envelope and within the nucleoplasm and

that exchanges occur between these two pools [40], our data suggest that the lamin nucleoplasmic pools bind to SREBP1 and contribute to regulate its function.

Localization of SREBP1 at the intranuclear periphery is favored by overexpression of full-length prelamin A or lamin A at the nuclear envelope

Recruitment of the SREBP1 lamin-binding polypeptide to the intranuclear periphery by lamin variants could be tested because, in the large majority of transfected cells, SREBP1 polypeptide overexpression did not cause the formation of SREBP1 aggregates and full-length lamin variants were incorporated into the lamina. As expected, there was an accumulation of unprocessed prelamin A L647R, which is mutated in the final Zmpste24 cleavage site and thus keeps the 15 farnesylated C-terminal amino acids, when this variant was expressed in cells. In contrast, wild-type prelamin A did not accumulate when it was overexpressed, indicating that its processing machinery including the enzymes isoprenylcysteine carboxymethyl transferase and Zmpste24 [5,41] was not saturated. In this context, our data revealed that unprocessed prelamin A, lamin A generated through prelamin A processing and lamin A expressed directly as a mature protein are able to associate into complexes with the SREBP1 polypeptide *in vitro* and *in vivo* and increase the recruitment of the SREBP1 polypeptide to the intranuclear periphery of cells. This supports a model in which nuclear envelope sequestration of SREBP1 results from a direct interaction of unprocessed and mature A-type lamins with SREBP1. In contrast, Capanni and co-workers could only co-immunoprecipitate SREBP1 with prelamin A but not with lamin A [12]. This discrepancy may be explained by the fact that lamins are expressed at different levels and within distinct cellular contexts. We used transfected HeLa cells in which lamins were overexpressed whereas Capanni et al. [12] used human fibroblasts or mouse preadipocyte cell line in which lamins were likely expressed at lower levels. In addition, we used a lamin-binding SREBP1 polypeptide instead of full-length mature SREBP1 [12]. Our interpretation is that in the cellular models of Capanni and co-workers [12], the SREBP1 binding motif although present in prelamin A, lamin A and lamin C is accessible only in prelamin A. We speculate that exposure of the SREBP1 binding motif on A-type lamins may change in relation to the relative protein level of the different lamin isoforms and/or in cells of different origins. We observed that proper exposure of the SREBP1 binding site likely existed in native HeLa cells, as 18% to 36% of these cells expressing only endogenous lamins concentrate the lamin-binding polypeptide of SREBP1 at the nuclear periphery. We hypothesize that SREBP1 recruitment at the nuclear periphery depends on the composition of the nuclear lamina and its organization. Although the lamina network is highly stable, the notion that it could rearrange agrees with observations in cells treated with inhibitors of prelamin A processing or subjected to mechanical stress [41,42].

Both wild-type and disease related variants of A-type lamins participate to the regulation of SREBP1 localization

We show that prelamin A R482W, expressed in FPLD, and progerin, expressed in HGPS, both bind the SREBP1 polypeptide *in vitro* and *in cells* and have an impact on the SREBP1 polypeptide intranuclear distribution in co-transfected cells. Our data agree with the previously formulated hypothesis that progerin actively contributes to the development of adipose tissue defects observed in progeria patients through its capacity to bind/sequester SREBP1 at the nuclear envelope [43]. In addition, the apparent similar impact of overexpressing either wild-type, FPLD or progeria associated lamin A variants on the sequestration of the lamin-binding SREBP1 polypeptide is in agreement with previous results showing that overexpression of either lamins WT, R482W or progerin have the same impact on adipocyte differentiation [44,45].

In conclusion, our results show that different A-type lamin variants have similar SREBP1 binding efficiencies and similar impacts on SREBP1 subnuclear distribution. However, the impact of these different lamins on the transcriptional activity of SREBP1 remains to be deciphered. As the SREBP1 polypeptide from amino acid 227 to 487, which binds to A-type lamins, contains the SREBP1 DNA binding domain, lamins could compete with DNA for SREBP1 binding. In the case of the lamin B1 / Oct-1 interaction, electrophoretic mobility shift assay and chromatin immunoprecipitation has revealed that Oct-1 binding to its targeted genes is enhanced in cells lacking functional lamin B1 [46]. Similarly, SREBP1 binding to its targeted genes could be hindered by A-type lamins and its disease-related variants. SREBP1 transcriptional activity would thus be regulated by the composition of the nuclear lamina, in particular the A-type lamin protein level, as well as the lamina's spatial organization, especially the A-type lamin accessibility. Lamin mutations causing FPLD and HGPS could contribute to SREBP1 misfunction by modifying lamin expression level, stability and accessibility.

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FIGURE 1. A polypeptide containing amino acids 227 to 487 of SREBP1 binds *in vitro* to the C-terminal regions of lamin A and lamin C

A) Schematic representation of the lamin tails fused to GST prepared for the *in vitro* binding experiments. Locations of portions 1a, 1b and 2 of the rod domain and the Ig fold are shown on the representations of the entire prelamins A and B1. Amino acid numbers are given above the diagrams of the lamin GST fusion proteins. The A-type lamin GST fusion proteins contain the lamin A tail from amino acid 389 to 646 (GST-cter LA), the lamin C tail from amino acid 389 to 572 (GST-cter LC) and the Ig fold domain common to lamin A and lamin C from amino acid 411 to 553 (GST-Ig fold). The lamin B1 fusion protein contains the lamin B1 tail from amino acid 391 to 586 (GST-cter LB). B) Schematic representation of the His-tagged SREBP1a fragment used in the *in vitro* binding experiments. A diagram of mature SREBP1a (SREBP1) with its acidic, Pro/Ser-rich and bHLH-zip domains is shown and amino acid numbers are given above. The His tagged fusion protein contains amino acids 227 to 487 of SREBP1. C) Purified SREBP1 polypeptide (His-SREBP1(227-487)) analyzed by SDS-PAGE and revealed by Coomassie blue staining. Molecular mass standards are shown on the right and their masses are indicated in kDa. D) Binding of the SREBP1 polypeptide to the lamin C-terminal regions assessed by GST pull-down. After SDS-PAGE and protein transfer onto nitrocellulose, the amounts of GST-lamin fusion

proteins were assessed by Red ponceau staining (upper panel), while bound SREBP1 polypeptide (His-SREBP(227-487)) was detected by immunoblotting using an anti SREBP1 antibody (lower panel). Migrations of molecular mass standards are indicated in kDa in the upper panel. E) Enhanced chemiluminescence (ECL) signals obtained for SREBP1 (His-SREBP1(227-487)), as shown in the lower panel of panel D, were scanned. The signal obtained with GST alone (GST^o) was considered to be non-specific and subtracted from all other signals. The highest signal obtained when GST pull-down was performed with GST-lamin C C-terminal region was used as the 100% reference. Bars correspond to the mean values of n = 4 independent experiments; vertical bars indicate s.e.m. and ** indicates statistically significant differences versus the signal obtained for GST-lamin C C-terminal region with p<0.001. F) ITC analysis of the interaction between the SREBP1 polypeptide (His-SREBP1 227-487) and the C-terminal region of lamin C (cter-LC).

FIGURE 2. The SREBP1 polypeptide fused to a nucleolar localization sequence is delocalized after overexpression of the C-terminal regions of lamin A and lamin C in cells

A) HeLa cells were mono-transfected to express either a FLAG-tagged construct of the lamin A tail from amino acid 389 to 646 (cter-lamin A), a FLAG-tagged construct of the lamin C tail from amino acid 389 to 572 (cter-lamin C), a GFP fusion of the SREBP1 polypeptide from amino acid 227 to 487 (SREBP1), a GFP fusion of the same SREBP1 polypeptide and containing a nucleolar localization sequence (Nols-SREBP1), or a GFP fusion of the nucleolar localization sequence (Nols). B) HeLa cells were co-transfected to express simultaneously GFP-Nols-SREBP1 (227-487) and either FLAG-cter-lamin A or FLAG-cter-lamin C. C) HeLa cells were co-transfected to express simultaneously GFP containing a nucleolar localization sequence (Nols) and either FLAG-cter-lamin A or FLAG-cter-lamin C. In all cases, 24 h post-transfection, cells were fixed in paraformaldehyde solution and GFP chimeric proteins were detected directly (GFP) while tagged-lamins were detected by immunofluorescence labeling with mouse anti FLAG antibodies (FLAG) and the nucleoli were revealed with human anti fibrillarin antibodies (Fibr.). DNA was stained with Hoechst dye. Note that upon expression of cter-lamins, GFP-Nols-SREBP1(227-487) accumulates predominantly either in nucleoli (arrows) or diffusely in the nucleoplasm (arrowheads). Bars correspond to 10 μ m. D) Quantitative analysis performed on cells co-transfected with vectors coding for either GFP-Nols-SREBP1(227-487) or GFP-Nols together with either an empty vector or vectors coding for the FLAG-tagged tail of lamin C (+ cter-LC) or the FLAG-tagged tail of lamin A (+ cter-LA), as indicated. The ordinate corresponds to the percentages of cells whose GFP signal accumulates in nucleoli. Data are the mean values of $n = 3$ to $n = 5$ independent experiments, as indicated (nber exps). Vertical bars indicate the s.e.m. and asterisks indicate statistically significant differences versus the signal obtained for [GFP-Nols-SREBP1(227-487) + empty vector], with * $p < 0.01$ and ** $p < 0.001$.

FIGURE 3. A-type lamin variants that occur in FPLD (R482W) or HGPS (Δ 607-656) do not prevent binding of the SREBP1 polypeptide to the tails of lamin A or prelamin A *in vitro*

A) Schematic representation of the prelamin A (preLA) tail sequence showing the location of amino acid substitution R482W (FPLD) and of the deleted amino acids 607-656 in progerin (HGPS). Locations of portions 1a, 1b and 2 of the rod domain and the Ig fold are shown on a representation of the entire prelamin A. Amino acid numbers are given above the diagrams. B) Binding of the purified GST-fusion proteins to the SREBP1 polypeptide (His-SREBP1(227-487)) was assessed by pull-down. GST-lamins and His-SREBP1(227-487) were detected as described in legend of Fig. 1. ECL signals obtained for SREBP1 (middle panel) were scanned (lower panel). The signal obtained with GST⁺ was considered as the minimal background and was subtracted from the other experiments, while the signal observed with GST-cter LA was referred as 100%. Note that the signal obtained with GST-cter preLA (607-656) was even weaker than the signal observed with GST⁺. Data are the mean values of $n = 4$ independent experiments; vertical bars indicate the s.e.m. The SREBP1 signals recovered with GST-cter preLA and GST-cter preLA R482W were not significantly different ($0.05 < p < 0.10$).

FIGURE 4. FLAG-prelamin A, FLAG-lamin A as well as disease-associated lamin variants R482W and progerin co-immunoprecipitate with the SREBP1 polypeptide in HeLa cells HeLa cells were transfected to express HA-SREBP1(227-487) alone or together with either FLAG-prelamin A WT, FLAG-lamin A, FLAG-prelamin A R482W, FLAG-prelamin A L647R and FLAG-progerin, as indicated in the table in the lower panel. Twenty-four hours post-transfection, lysates were prepared (see materials and methods) and used for immunoprecipitations, using mouse anti FLAG antibodies (IP α FLAG) or mouse unrelated antibodies (IP α GFP). Proteins present in the lysates (Input; lanes 1, 2, 7, 10, 11 and 16-19) and in the immunocomplexes (IP; lanes 3-6, 8-9, 12-15 and 20-26) were separated by electrophoresis, western blotted and immunoprobed with either rabbit anti FLAG antibodies (Ibnot α FLAG) or rabbit anti SREBP1 antibodies (Ibnot α SREBP1), as indicated. Note the co-immunoprecipitation of HA-SREBP1 (227-487) with FLAG-(pre)lamin A WT (lanes 6, 13 and 25), FLAG-lamin A (lane 9), FLAG-(pre)lamin A R482W(lane 15), FLAG-(pre)lamin A L647R (lane 21) and FLAG-progerin (lane 23).

FIGURE 5. The lamin-binding polypeptide of SREBP1 shows three patterns of nuclear distribution

Hela cells were transfected to express the HA-tagged lamin-binding polypeptide of SREBP1 (HA-SREBP1(227-487)). Twenty-four hours after transfection, cells were fixed in paraformaldehyde solution either directly or following pre-extraction with non-ionic detergents and salt (see Materials and Methods). A) Fluorescence micrographs showing Hoechst dye staining of nuclei (DNA) or immunofluorescence performed using mouse anti HA antibodies (HA-SREBP1(227-487)) with overlaps of these signals shown in the right panels (DNA SREBP1). B) Fluorescence micrographs showing nuclear staining with Hoechst dye (DNA) and double-immunofluorescence labeling with mouse antibodies against HA (HA-SREBP1) and rabbit antibodies against lamins A and C (Lamin A/C) without (upper panels) and after (lower panels) extractions with non-ionic detergents and salt. Overlaps of the HA and lamin signals are shown in the right panels (HA-SREBP1 Lamin A/C). Note the localization of the SREBP1 polypeptide either in the nucleoplasm (Nu; upper panels in A), at the nuclear periphery (Pe; middle panels in A, and all panels in B), or within aggregates (Ag; lower panels in A). The co-localization of the SREBP1 polypeptide with lamins A and C at the nuclear envelope can be appreciated in panel B. Bar = 10 μ m.

FIGURE 6. Overexpression of wild-type prelamin A, lamin A and disease-associated lamin variants has an impact on the subnuclear localization of the lamin-binding polypeptide of SREBP1

A) HeLa cells were transfected to express FLAG-tagged full-length wild-type prelamin A ((pre)LA WT), prelamin A R482W ((pre)LA R482W), progerin, lamin A (LA), prelamin A L647R (preLA L647R), GFP-lamin B1 (LB), or a FLAG-tagged tail-deleted form of lamin A (Lamin 1-431). B-H) HeLa cells were co-transfected to express the HA-tagged lamin-binding polypeptide of SREBP1 (SREBP1) with either FLAG-tagged full-length (pre)LA WT (B), FLAG-tagged full-length (pre)LA R482W (C), FLAG-tagged full-length lamin 1-431 (D), GFP-LB (E), FLAG-tagged full-length LA (F), FLAG-tagged full-length preLA L647R (G), or FLAG-tagged progerin (H). Twenty-four hours after transfection, cells were fixed in paraformaldehyde solution and immunofluorescence microscopy performed using either mouse anti FLAG antibodies alone (all panels in A except GFP-LB), rabbit anti SREBP1 antibodies alone (E), or rabbit anti SREBP1 antibodies together with mouse anti FLAG antibodies (B-D, F-H). Nu, Pe and Ag refer to the three distinct SREBP1 phenotypes as indicated in the legend of Fig. 5. Bar = 10 μ m. (I-J) Quantitative analysis of the localization of the HA-tagged lamin-binding polypeptide of SREBP1 upon overexpression of FLAG tagged full-length wild-type and variant forms of prelamin A. The ordinate corresponds to the frequencies of three SREBP1 phenotypes: detection in nucleoplasm (Nu), at the periphery within nuclei (Pe), within nucleoplasmic aggregates (Ag). Data are the means of 2 to 6 independent experiments as indicated (nber exps). Vertical bars indicate the s.e.m.. Note the increased nuclear peripheral accumulation of the HA-tagged lamin-binding polypeptide of SREBP1 upon overexpression of full-length prelamins (WT and R482W), lamin A and progerin.

FIGURE 7. Prelamin A accumulates in cells expressing FLAG-prelamin A L647R

A) Protein extracts of HeLa cells mock transfected (lanes 1) or transfected to express either FLAG-tagged full-length prelamin A L647R (lanes 2) or FLAG-tagged full-length wild-type prelamin A (lanes 3 and 3') or FLAG-tagged full-length lamin A (lane 4) were separated by SDS-PAGE, transferred to nitrocellulose and probed with antibodies directed against prelamin A (G α preLA, left panel) or FLAG (M α FLAG, right panels) or B) HeLa cells were transfected to express the HA-tagged lamin-binding polypeptide of SREBP1 (SREBP1(227-487)) alone (-) or together with either FLAG-tagged full-length prelamin A L647R (+ preLA L647R) or FLAG-tagged full-length wild-type prelamin A (+ (pre)LA WT) or FLAG-tagged full-length lamin A (+ LA). Twenty-four hours post-transfection, cells were fixed in paraformaldehyde solution and double immunofluorescence microscopy was performed using goat anti prelamin A antibodies with either rabbit anti FLAG or rabbit anti SREBP1, as indicated. In the displayed cells, the HA-tagged lamin-binding polypeptide of SREBP1 accumulates at the intranuclear periphery (Phenotype Pe). Note that only cells expressing Flag-prelamin A L647R have intense prelamin A labeling at the nuclear envelope (arrows). Bar = 10 μm.