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Research Article

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 of (R482W) and Hutchison Gilford progeria syndrome (Δ607–656) 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 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.

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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 AΔ10 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 aging [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-Giford 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 PPARγ 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 BamH1 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 BamH1 and EcoRI sites of pGEX-2T. The GST fusion protein containing the sequence deleted in the progerin (amino acids 607–656) was obtained

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