Targeting lamin proteins to the nuclear envelope: the role of CaaX box modifications

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Introduction

The nuclear lamina is a protein network lining the nucleoplasmic surface of the inner nuclear membrane. It is presumed to represent a karyoskeletal element important for nuclear envelope integrity and interphase chromatin organization (for reviews see [1, 2]). Its major constituents, the nuclear lamins, are members of the intermediate filament (IF) protein family [3-5]. On the basis of biochemical properties, structural criteria, and expression patterns, vertebrate lamins have been classified as either A- or B-type. Two structurally distinct B-type lamins (B₁ and B₂) are expressed in virtually all somatic cells, but expression of A-type lamins is developmentally regulated, with little or no lamin A being expressed in undifferentiated cell types (reviewed in [6]). During mitosis the nuclear lamina is transiently disassembled in response to hyper-phosphorylation of lamin proteins [7-10], and recent results implicate the p.34+[N] protein kinase in directly controlling this process [11-13]. While B-type lamins remain associated with membranes throughout the cell cycle, A-type lamins are found in a soluble state in mitotic cells [7, 14].

Lamins share with cytoplasmic IF proteins a tripartite organization in that they display a central α-helical rod domain, flanked by non-α-helical domains at the N- and C-terminal ends. However, lamins are readily distinguished from cytoplasmic members of the IF family by the presence of a nuclear localization signal (NLS) and a C-terminal CaaX box (reviewed in [6, 15]). The former motif is necessary for nuclear transport [16], while the CaaX box is modified by isoprenylation, proteolytic trimming, and carboxyl methylation [17-19]. Such modifications occur not only on lamins, but also on certain yeast mating factors, ras proteins and many other GTP-hydrolysing (G-) proteins (reviewed in [20-23]). These hydrophobic modifications appear to represent a general mechanism for increasing the affinity of proteins for various types of cellular membranes [24, 25]. In this article, we briefly discuss the functional significance of CaaX box modifications for targeting lamins to the nuclear envelope.

Post-translational processing and assembly of newly synthesized lamins

Post-translational processing of lamin proteins had been discovered several years before the identification of the CaaX box as a target for isoprenylation and carboxylmethylation [26-29]. While studying the biosynthesis of chicken nuclear lamins, A, B₁ and B₂ by pulse-chase experiments, we found that newly synthesized lamin B₁ was rapidly converted into a product with higher electrophoretic mobility [29]. The lamin B₁ precursor displayed a half-life of approximately 2-3 min, and its processing thus occurred with similar kinetics as the nuclear uptake of the newly synthesized protein [29]. Lamin A was also converted into a mature form displaying higher electrophoretic mobility, but processing of the lamin A precursor was considerably slower and occurred only after incorporation of the newly synthesized protein into the pre-existing nuclear lamina [29]. To further investigate the molecular nature of these distinct processing reactions, cloned cDNAs for lamins A and B₁, and B₂ were used for coupled transcription-translation experiments in vitro [30]. These studies confirmed that both lamins A and B₁ are synthesized as precursor proteins. However, whereas the lamin A precursor appeared to be stable in the reticulocyte lysate, the lamin B₁ precursor was processed to the mature-size protein even in this cell-free system [30]. These results confirmed that two distinct activities were responsible for the processing events that had been observed in vitro, but a molecular explanation had to await the identification of lamins as acceptors for isoprenoids [31, 32].

The reticulocyte lysate: a powerful in vitro system for studying polyisoprenylation

Prompted by the discovery that lamin proteins are isoprenylated in vitro [31, 32], we asked whether isoprenylation might conceivably account for the processing in vitro of lamin B₁. Although unex-
pected at the time, we found that in a reticulocyte lysate all three lamins, A, B, and B, readily incorporated a derivative of mevalonic acid, the precursor of isoprenoids [17]. This reticulocyte lysate assay provides a simple but powerful tool for studying the enzymology of various types of isoprenylation reactions. The reticulocyte cell-free system in fact supports the incorporation of at least two types of polyisoprenyl groups (farnesyl and geranylgeranyl) into proteins with appropriate C-terminal acceptor motifs [33-39]. Provided that microsomal membranes are added to the reticulocyte lysate, the system also carries out the proteolytic trimming and carboxyl methylation of isoprenylated proteins containing the CaaX-box [40].

**CaaX box modifications are required for assembly of newly synthesized lamins at the nuclear envelope**

To explore the significance of CaaX box modifications for the function of nuclear lamins, several laboratories have combined mutational analyses with either micro-injection or transfection experiments [19, 41, 42]. These studies concur to demonstrate that isoprenylation and carboxyl methylation are required for correct subnuclear targeting of newly synthesized lamins (for a schematic summary see Fig. 1). Moreover, these hydrophobic modifications were shown to be important for mediating the interaction of lamins with the nuclear membrane, rather than for nuclear localization of lamins, or for lamin polymerization per se (for further discussion, see [19]).

**Nuclear membrane association of lamins: CaaX box modification versus receptor binding**

Both A- and B-type lamins are isoprenylated and carboxyl-methylated shortly after synthesis. Subsequently, however, lamin A specifically undergoes an additional processing event (see Fig. 1). This occurs concomitant with, or shortly after, incorporation of the lamin A precursor into the pre-existing nuclear lamina [26, 29, 43]. Lamin A-specific processing consists of the proteolytic removal of some 18-20 amino acids from the C-terminus [17, 44]. This leads to the loss of the hydrophobically modified C-terminus from A- but not B-type lamins, and may help to explain why A-type lamins are solubilized during mitosis, while B-type lamins remain membrane bound.

Although it is tempting to speculate that B-type lamins are membrane-associated throughout the cell cycle because they carry a permanent hydrophobic modification at the C-terminus, detailed studies with ras proteins lead to the conclu-
sion that isoprenylation and carboxyl methylation per se do not confer a strong membrane association. Instead, efficient binding of ras proteins to the plasma membrane was shown to require additional palmitoylation of another cysteine, or the presence of a polybasic domain, in close proximity to the isoprenylated C-terminus [24, 25]. In the case of lamins, palmitoylation has not been detected, and, with the exception of the NLS, there are no conspicuous stretches of basic residues that might constitute functional counterparts of those characterized in p21 proteins. Thus, it remains to be determined to what extent isoprenylation and carboxylmethylation alone are sufficient to explain the membrane association of B-type lamins.

Mitotically disassembled lamins exist as dimers (or even higher oligomers) [7, 45]. Therefore, it is conceivable that the presence of multiple isoprene substituents might generate sufficient avidity to explain persistent membrane binding of B-type lamins (see Fig. 2a). Such a model is attractive since an anchorage via lipophilic modification would be expected to favour rapid lateral diffusion of B-type lamins in lipid bilayer membranes. This in turn might explain the observed extensive redistribution of lamin B, between mitotic remnants of the nuclear envelope and elements of the endoplasmic reticulum [14]. On the other hand, lamins clearly interact, either directly or indirectly, with integral proteins of the inner nuclear membrane. Several candidate 'lamin receptors' have been identified [46–50], although it is presently unclear how these proteins bind to lamins. According to one model, they may bind lamins via protein–protein interactions (Fig. 2b); alternatively, however, it is conceivable that they may function as farnesyl receptors (Fig. 2c). Also, it remains to be deter-

**Fig. 2**

**Hypothetical models describing the interaction between B-type lamins and the inner nuclear membrane**

According to model A, dimeric (or tetrameric) lamins are bound to the membrane exclusively via their isoprenylated and carboxyl-methylated C-termini. Models B and C invoke additional interactions with integral membrane proteins. Such 'lamin receptors' may bind either the lamin polypeptide (B) or conceivably the isoprene substituents (C).
Fig. 3), mature lamin A interacts with the surface of condensed chromosomes before reformation of the nuclear envelope. Thus, in contrast to the lamin A protein that is synthesized during interphase of the cell cycle, mitotic lamin A may benefit from direct access to as yet unidentified binding sites at the chromosome surface. Polymerization at the chromosome surface may then provide an alternative (CaaX-independent) assembly pathway for nuclear lamin proteins. Such a post-mitotic assembly mechanism may also explain how lamin C accumulates at the nuclear envelope. Lamin C is unique in that it lacks a CaaX box. It presumably arises from differential splicing of the mammalian lamin A gene, but so far there is no evidence for functional differences between laminins A and C [5].

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