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Maturation of nuclear lamin A involves a specific carboxy-terminal trimming, which removes the polyisoprenylation site from the precursor; implications for the structure of the nuclear lamina

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Lamin A, a nuclear lamina protein of differentiated cells, is synthesized as a precursor of the mature molecule. Protein sequencing of the carboxy-terminal 14 kDa fragment shows a lack of the last 18 residues predicted by cDNA sequencing. The carboxy-terminal proteolytic maturation explains previous biochemical results including the loss of the polyisoprenylation site now located to the CXXM motif at the end of the chain. This view and earlier results on lamin B predict multiple post-translational modifications shared by lamins A and B. While retained by lamin B, which is present in all cells, they are lost by maturation from lamin A, which probably acts only as an additional lamina constituent in differentiated cells.

Isoprenylation; Lamin; Protein modification; Proteolyis; Protein ras

1. INTRODUCTION

The nuclear lamina lining the nucleoplasmic surface of the inner membrane is important for organization of interphase chromatin and envelope integrity (for reviews see [1,2]). Its major structural components are the lamins. Mammalian lamins A and C arise by differential splicing from the same transcript with the smaller lamin C essentially being a truncated form of lamin A [3,4], while lamin B is encoded by a different gene [5]. Birds express lamin A and two distinct lamin B species but no lamin C [6,7]. Although all cDNA sequences for lamins A and B predict the same carboxy-terminal motif CXXM with X being preferentially an aliphatic-hydrophobic residue, the larger lamin A molecules carry a unique domain which precedes the CXXM motif [4–11]. During mitosis the lamina disassembles probably due to hyperphosphorylation. Lamin A-type molecules become solubilized while B-type lamins remain associated with membrane vesicles [12]. Metabolic labelling studies and pulse-chase experiments point to multiple post-translational steps between the synthesis of lamin polypeptide chains on free polysomes and their mature forms which are deposited into the lamina [13–20]. Lamin B-type molecules become *O*-methylated [16–18] and incorporate a very hydrophobic derivative of mevalonic acid, thought to be a polyisoprenylate group [19,20]. Mature lamins A of mammals and chicken show a reduction in apparent molecular weight on SDS gels [13,14,20] and become less basic upon isoelectric focussing [14]. To understand the transition between the lamin A precursor and the postulated mature lamin A in a background of other modifications requires the sequence predicted from the cDNA to be matched to the amino acid sequence established by direct sequencing of the mature lamin A molecule.

Here we show by protein sequencing that lamin A isolated from EAT cells lacks the carboxy-terminal 18 residues predicted from the cDNA sequence. The postulated proteolytic processing event removes the CXXX sequence at the carboxy-terminal end, which is thought to be the site of polyisoprenylation. Thus mature lamin A lacks the membrane anchor retained by mature lamin B. This molecular difference between mature lamins A and B nicely fits the observation that all cells express lamins B, while lamin A expression seems an additional event reserved to cells of differentiated tissues [21–23].

2. MATERIALS AND METHODS

Lamins were purified from the murine EAT cell line as described [24]. Fractions containing a mixture of lamins A and C were used (fig.1). After reaction of the cysteines with 2-vinylpyridine the protein was subjected to cleavage with BNPS-skatole [25]. Fragments were separated by HPLC using a PLRP-S column (300 Å, 8 μm; 150 × 4.6 mm; Polymer Laboratories) and an acetonitrile gradient in 0.1% trifluoroacetic acid. The carboxy-terminal 14 kDa BNPS-skatole fragment of lamin A was identified by its N-terminal sequence. This followed the amino acid sequence past the last tryp-

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Fig.1. SDS-PAGE of lamins A plus C isolated from murine EAT cells (lane 2). Lane 1 gives standard proteins and the arrowhead marks the top of the gel.

tophan predicted from the cDNA sequence (fig.2a). Gas phase sequencing was on an Applied Biosystems sequenator (model A470) with an on-line PTH amino acid analyzer. The remainder of the sequence of the fragment was established from peptides obtained by digestion with trypsin and endoproteinase Asp-N, respectively. Peptides were separated by HPLC on a Shandon C-18 column and sequenced. The shorter carboxy-terminal BNPS-skatole fragment of lamin C was also identified. Its sequence was obtained by automated sequencing of the fragment and the peptides arising from digestion with trypsin and endoproteinase Glu-C, respectively.

3. RESULTS

To map the molecular difference between the lamin A precursor and the mature lamin A, we used the mixture of lamins A and C from murine EAT cells (for purity see fig.1). Lamin A molecules from man, chicken and *Xenopus* [4,6,9] show high sequence homology and conserve 4 tryptophan residues in the carboxy-terminal tail domain. The last of these tryptophans (position 520 of the human sequence) lies some 145 residues before the carboxy-terminal end predicted for the lamin A precursor. Therefore the mixture of murine lamins A and C was treated with BNPS-skatole. The carboxy-terminal fragment of lamin A was characterized by its N-terminal sequence and the se-

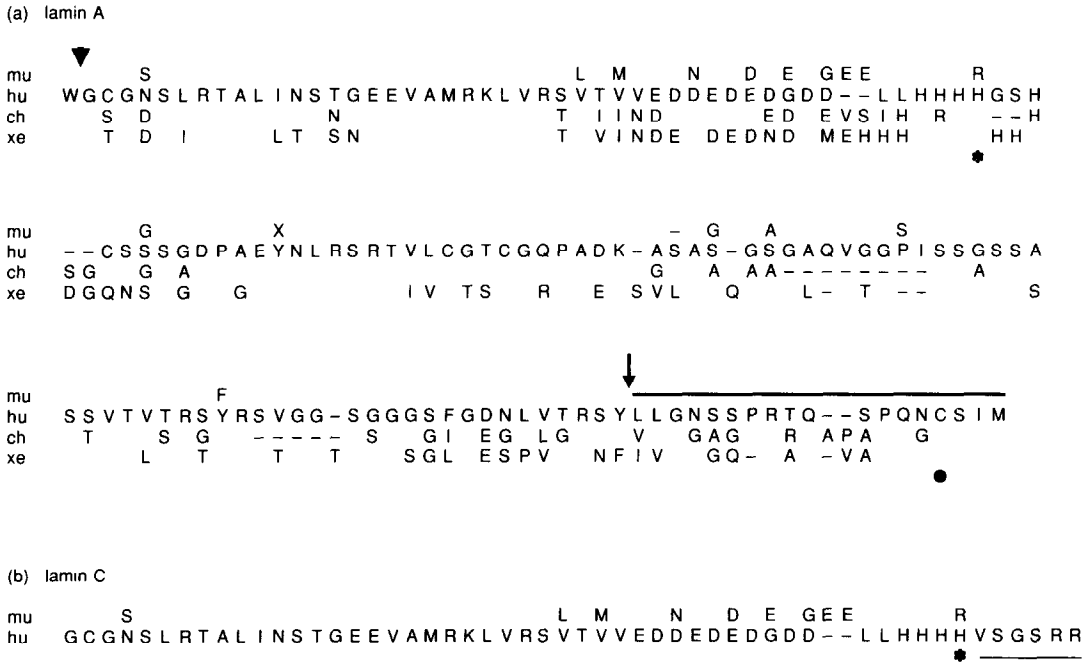

Fig.2. (a) Carboxy-terminal sequences of the lamin A precursors predicted by cDNA sequencing for man (hu), chicken (ch) and *Xenopus laevis* (xe) [4,6,9]. In the chicken and *Xenopus* sequences only those residues differing from the human sequence are given. Dashes allow for a better alignment. The upper line (mu) gives the corresponding sequence for the mature murine lamin A established by protein chemistry. Again only changes versus the human sequence are given. The sequence starts past the last tryptophan (W) at which BNPS-skatole cleaves (marked by an arrowhead; residue 520 of the human sequence [4]). Note that the carboxy-terminal 18 residues predicted from the cDNA for the human precursor are absent in the mature murine protein (thick line). The arrow marks the proposed proteolytic site. Note also the presence of several cysteine residues (C) earlier in the 14 kDa region and the single cysteine in the region lost upon maturation. The latter is marked by a filled circle to indicate where the precursor was polyisoprenylated (see section 4). The residue marked X in the murine sequence indicates a potential microheterogeneity in lamin A of EAT cells as in different peptides it was encountered as Y and C respectively. (b) Murine lamin C shows the same sequence as lamin A up to the R marked by a star in panels (a) and (b). The subsequent 6 residues are unique to lamin C (underlined). The panel shows a comparison of the carboxy-terminal region of murine lamin C obtained by protein chemistry with that of human lamin C predicted from the DNA sequence [3,4].

quences obtained on tryptic peptides and peptides derived by endoproteinase Asp-N. As shown in fig.2a the last 18 amino acid residues predicted by the human cDNA sequence were absent in the completed sequence of the carboxy-terminal fragment of lamin A. The lack of lysine and arginine in the tryptic peptide SY strongly suggested that this dipeptide corresponds to the carboxy-terminal end of mature lamin A. This positioning was confirmed by the overlapping octapeptide DNLVTRSY obtained by endoproteinase Asp-N, which cleaves before Asp (fig.2a). Maturation of lamin A therefore involves a specific proteolytic event occurring 18 residues from the carboxy-terminal end. The residues adjacent to the cleavage site are conserved in the 3 lamin A precursors for which cDNA sequence is available. The sequence preceding the site is RSY in mouse, man [4] and chicken [6], and RNF in *Xenopus* [9]. In the 3 species for which the sequence is known, the 3 residues following the site are XXG, where X is a large hydrophobic residue [4,6,9] (see fig.2a).

As a further consequence of our analysis we provide the sequence of a 14 kDa fragment of murine lamin A and show that over this region murine and human lamin A display 88% sequence identity (fig.2a). The results on murine lamin C show that just as in man [3,4] this lamin has only 6 unique residues at the carboxy-terminus, which are not found in lamin A. Lamin C is not subjected to maturation since the protein sequence follows the prediction from the cDNA sequence (fig.2b).

4. DISCUSSION

We have shown by protein sequencing that mature murine lamin A lacks the carboxy-terminal 18 residues predicted by the cDNA sequence of the human lamin A precursor. Thus the lamin A maturation observed by others [13–15] involves single or multiple proteolytic events at the carboxy-terminal end of the precursor. The proposed final cleavage site shows a reasonably strong homology in the currently available precursor sequences. The position of the cleavage site is consistent with earlier results [13,14] indicating a change in apparent molecular mass of some 2000 to 3000 and a more basic p*I* for the precursor [14] (see fig.2a). Since maturation is thought to be a nuclear process [13,14] a nuclear protease seems responsible for the event. The position of the cleavage site also fits the recent observation by Beck et al. that polyisoprenylation of the lamin A precursor is lost on maturation [20]. This result together with the cleavage site, now defined, locates the isoprenylation site as a cysteine specific modification [26–28] to the sole cysteine in the last 18 residues of the lamin A precursor. This cysteine is part of the carboxy-terminal CXXM motif (fig.2a).

Studies on yeast *a* factor [26] and ras proteins [27] show that a carboxy-terminal CXXX sequence (X being predominantly an aliphatic-hydrophobic residue) is involved in the following set of post-translational modifications: polyisoprenylation at the sulfur of the cysteine, removal of the three X residues and methylation of the new carboxylate of the cysteine (our interpretation of the order of events). The polyisoprenyl substitution seems to be the farnesyl$C_{15}H_{25}$ group [26,28]. A similar scenario is at least in part already indicated for the mature lamin B forms, which again carry the CXXM motif [5–8,10,11]. Mature lamin B is methylated [16–18] and contains a stable polyisoprenyl substitution [19,20]. Thus the multiply modified carboxy-terminal cysteine of mature lamin B becomes a perfect membrane anchor for lipid insertion. The loss of such an anchor in mature lamin A explains its solubilization in mitosis where lamin B remains membrane bound [12,29]. Thus lamin C, which lacks a possible modification site (fig.2b), and lamin A, which loses the substitution upon maturation (fig.2a), differ from lamin B in that they lack a membrane anchor and therefore probably cannot build a nuclear lamina on their own. This view fits the finding that lamin B expression is constitutive in all cells, while lamins A/C when present are additional lamina components only found upon cell and tissue differentiation [21–23]. This leaves the physiological function of lamin A maturation open. Since lamin A seems to be processed like lamin B to the polyisoprenylated state but subsequently loses its potential anchor, further work should concentrate on testing whether the unique 60 residue carboxy-terminal domain of lamin A provides a possible interaction site for specific nuclear components. Since lamin B lacks this domain it may be involved in interactions specific for differentiated cells. Interestingly A-type lamins are thought to interact more directly with chromatin than lamins B [12]. It also remains to be seen why the protease responsible for lamin A maturation does not recognize lamin B.

During the review process we have learned that K. Vorburger, G.T. Kitten and E.A. Nigg (manuscript submitted to EMBO J.), using a different approach, have identified the cysteine in the CXXM motif as the isoprenylation site in chicken lamin B_2 and also in the precursor for chicken lamin A. We also note that a full-length clone for murine lamin C has meanwhile been sequenced [30]. Our protein sequence of the carboxy-terminal fragment of murine lamin C is identical to the sequence predicted from this cDNA. Thus lamin C is not subject to a carboxy-terminal maturation.

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