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Protein chemical analysis of purified murine lamin B identifies two distinct polypeptides B1 and B2

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Lamin B purified from murine EAT cells was characterized by partial protein sequences. Contrary to the current view that mammals express only a single lamin B polypeptide corresponding to a characterized murine cDNA clone, our analysis documents two distinct B lamins. One protein follows the estabished cDNA sequence while the other identifies a novel murine lamin B. Comparison with the two chicken lamin B sequences established by cDNA cloning identifies the first murine lamin B sequence as a B1 type and the second as a B2 type. We conclude that mammals express two distinct lamin B forms as established by others for chicken.

Differentiation; Lamin; Multigene family; Nuclear lamin; Nucleus

1. INTRODUCTION

The nuclear lamina is a karyoskeletal structure which lines the nucleoplasmic side of the inner nuclear membrane. It is thought to be involved in nuclear envelope integrity, organization of interphase chromatin and the anchorage of the nuclear pore complexes (for reviews see [1,2]. The major structural proteins of the nuclear lamina are called lamins [3]. Traditionally only 3 lamin polypeptides are defined for various tissues of different mammals [1-5]. cDNA cloning has shown that mammalian lamins A and C, which differ only in their carboxyterminal region, most likely arise by differential RNA processing [6,7] while lamin B is encoded by a separate gene [5]. In contrast, gel electrophoretic and immunological results on chicken cells defined two B lamins (B1 and B2) and a single lamin A [8]. Although the latter work raised the possibility of a polymorphism of mammalian B lamin, the conservative view has been the assumption of a single mammalian B species [1-5], since the avian lamin B2 was sometimes thought to reflect an A-type lamin [1]. In spite of this difference there has been agreement on a differential expression of lamin A versus a general constitutive expression of lamin(s) B during chicken and mammalian embryogenesis [9-11]. As the recent rigorous proof for the existence of two avian B lamins by cDNA cloning [12,13] opened again the problem of a lamin B heterogeneity for mammals, we have turned to a protein chemical analysis of murine lamin B. Ehrlich ascites (EAT) cells propagated in mass culture are an excellent source for the purification of murine lamins suitable for biochemical and protein chemical studies [14]. We have earlier used the lamin A from this source to show that the postulated maturation of the lamin A precursor [15-17] involves a proteolytic trimming, which removes the carboxyterminal 18 residues including the isoprenylation site situated at the cysteine in position 4 from the end of the sequence predicted by cDNA cloning for the precursor [18] (see also reference [19]). We now make use of the lamin B fraction purified from EAT cells. Partial protein sequencing of CNBr fragments unambiguously shows the presence of two B subtypes. Thus mammals show the same lamin B polymorphism as chicken. The two murine lamin B sequences closely follow those predicted for chicken lamins B1 and B2 by cDNA cloning [12,13].

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2. MATERIALS AND METHODS

Lamin B was purified from murine EAT cells as described [14]. After alkylation of its cysteine residues with 2-vinyl-pyridine, cleavage with CNBr was by standard procedures. The fragment mixture was subjected to HPLC on a Vydac C18 column using a gradient from 5% to 60% acetonitrile in 0.1% trifluoroacetic acid. Fragments were characterized by automated sequencing using an Applied Biosystems gas phase sequencer (model A470) and a Knauer sequenator (model 810). Both instruments were equipped with an online PTH-analyzer. Some CNBr fragments and some mixtures of such fragments were also subjected to digestion with endoproteinase Lys-C, which cleaves at the C-terminal side of lysine. Resulting digests were processed through C_{18} chromatography and sequencing as above. Given the specificity of the CNBr cleavage we assume in the sequence summary that N-terminal sequences established on CNBr fragments are preceded by a methionine residue (for the N-terminal fragment see below). Similarly, given the high specificity of endoproteinase Lys-C, we assume that a lysine precedes an interior sequence of the enzymatically derived peptides obtained from CNBr fragments.

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3. RESULTS

Fig.1 documents the purity of the murine lamin B preparation used for CNBr cleavage. Peak fractions obtained by HPLC were subjected to extensive automated sequencing. In the case of some mixtures, the material was treated with endoproteinase Lys-C to obtain secondary peptides suitable for sequence analysis. The same approach was also used for few pure fragments which included a large fragment with a N-terminal blocking group (see below). Fig.2 summarizes the partial sequence data on murine lamin B. The sequences fall into two distinct groups. The first set follows unambiguously the lamin B sequence previously predicted from a murine cDNA clone [5]. The second set of sequences which covers 5 continuous stretches corresponding to a total of 171 residues is not contained in the predicted sequence. As shown in fig.2, these extra sequences arise from a novel lamin B type.

The presence of two distinct murine B lamins is readily understood when the sequences predicted for chicken lamins B1 and B2 by cDNA cloning [12,13] are inspected. The latter work already noted the high homology between the sequences of chicken lamin B1 and the earlier published murine lamin B [12], which we now identify as lamin B1. Since the novel murine lamin B sequences show a much higher homology with chicken lamin B2 than with lamin B1 from either mouse or chicken (fig.2) they clearly establish the B2 type of murine lamin.

There are two major reasons why our current analysis covers more sequences from lamin B1 than lamin B2. First, early in the work we encountered a large CNBr fragment with a N-terminal blocking group. Secondary cleavage with endoproteinase Lys-C established most of the sequence of this fragment and showed that it corresponds entirely to lamin B1 (positions 2–212). Second, for all 5 stretches of the lamin B2 sequence obtained we aimed at the corresponding sequence of lamin B1 as predicted by the only lamin B cDNA clone so far described [5] (see fig.2). The finding that the N-terminal CNBr fragment of lamin B1 retains the blocking group of the mature protein clearly indicates a N-terminal processing. Since CNBr cleaves past methionine the initiator methionine predicted in the B1 sequence as residue 1 (fig.2 and [5]) is not retained in the mature protein, since it an its N-terminal fragment carry a blocking group. Thus the initiator methionine is removed after protein synthesis and the subsequent alanine residue is most likely acetylated at its amino group. In agreement, we find by mass spectroscopy a molecular weight of 3519.5 for the N-terminal peptide obtained by endoproteinase Lys-C, while the sequence of residues 2–34 (see fig.2) plus an acetyl group predicts a value of 3517.9.

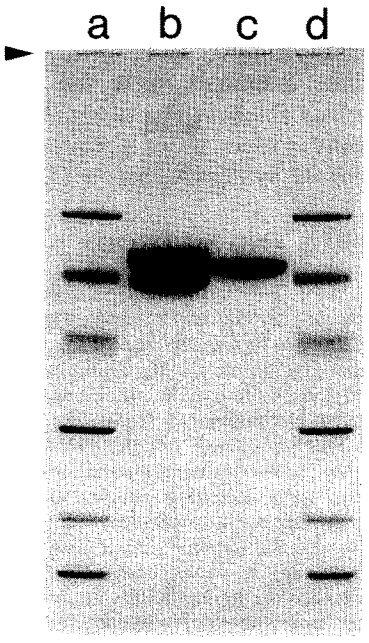

Fig.1. SDS-polyacrylamide gel electrophoresis of murine lamin B purified from EAT cells (lane c). Lane b shows the mixture of lamins A plus C. Lanes a and d give marker proteins. The arrowhead marks the top of the gel.

4. DISCUSSION

The direct protein-chemical proof that a seemingly homogeneous murine lamin B preparation contains two distinct polypeptides B1 and B2, each closely relatd to its counterpart in chicken, unifies our view on differential lamin expression in higher vertebrates. Thus contrary to the currently held view [1–5], mammals express two lamins B as already documented for chicken [8,12,13]. Our results on murine lamins emphasize again the need for standardized two-dimensional gels and for computerized databanks which become increasingly useful [20]. In reinspecting published two-dimensional gels of various lamin preparations, we note an abnormality for chicken B lamins. In spite of its higher molecular weights established by sequence [13], lamin B2 shows a lower apparent molecular weight than lamin B1 [9] while the relative separation in the other direction seems to reflect the true charge difference (our unpublished calculation). With this reservation we tentatively propose that a slightly more basic spot next to murine lamine B (B1), which on optimal gels has a slighly higher molecular weight than B1, is most likely the murine lamin B2 (see for instance fig.2 in [5]). If we allow for some abnormality of lamin B in the second direction and species-specific influences, the recently recognized additional minor lamin spots of rat liver could be B2 forms although they reach the apparent size of lamine A [21].

The documentation of two lamin B types for mouse has certain implications for lamin expression in murine embryogenesis. Previous studies on chicken [9] and

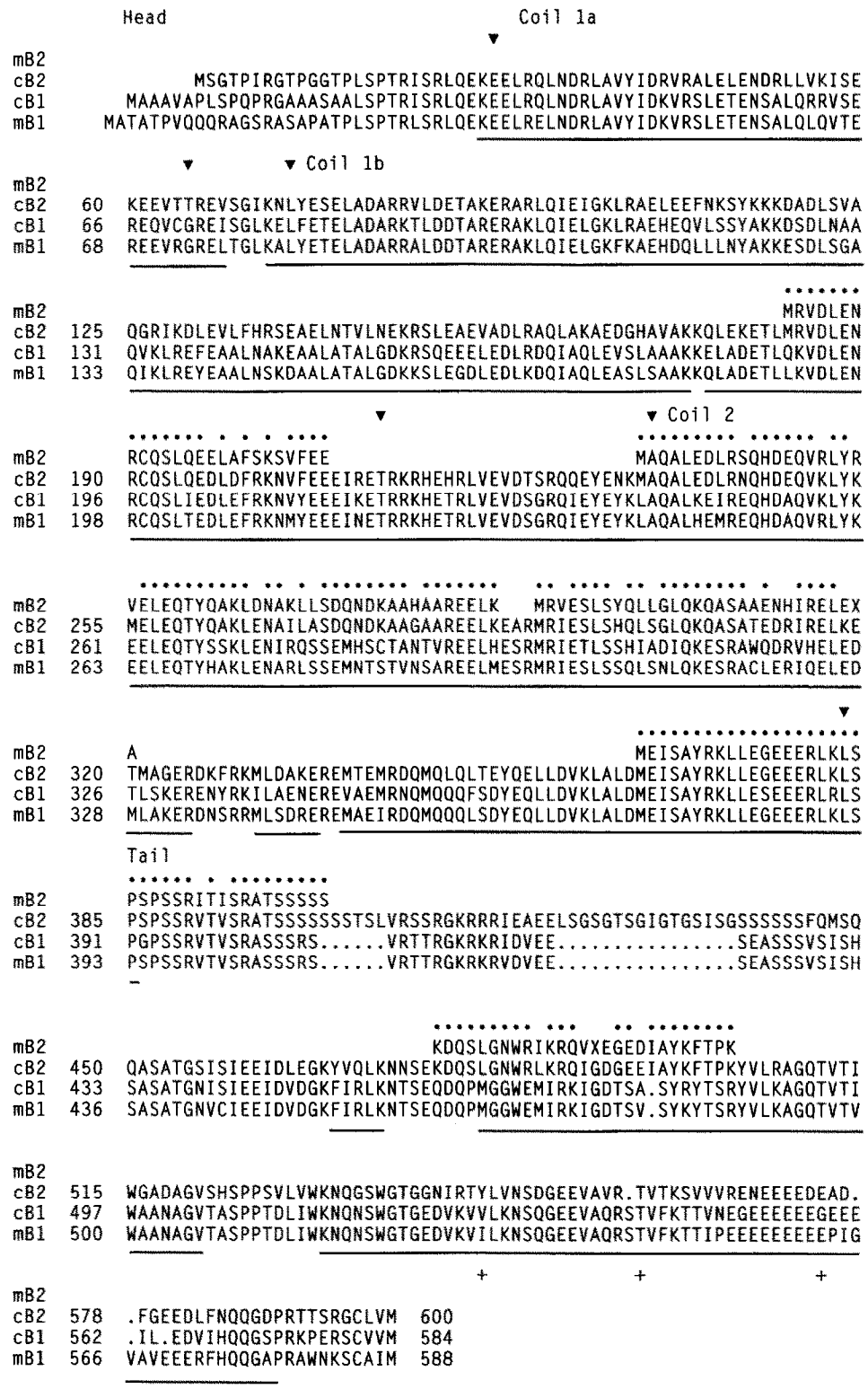

Fig.2. Alignment of the amino acid sequences predicted by cDNA cloning [5,12,13] for chicken lamin B2 (cB2), chicken lamin B1 (cB1) and a murine lamin B. This murine lamin B sequence is now identified as lamin B1 (mB1) (see section 3). The starts and ends of the coiled coil segments (coil 1a, 1b and 2) of the α-helical rod are marked by arrowheads. The small head domain and the large globular tail domain are also indicated. Sequences established by protein chemistry on murine lamin B are indicated either by lines below the murine lamin B sequence (mB1) or given above the chicken B2 type since they define the murine B2 type (mB2, see section 2). Note the high sequence homology between chicken lamin B2 and the newly defined murine lamin B2 (dots above the top line mark identical residues in both proteins). Note also that the sequence of murine lamin B1 so far established by protein chemistry differs at a few positions from that predicted by c-DNA cloning [5]. They are marked by a plus. The first two positions indicate a change in amino acid, the last plus marks an additional residue.

mouse [10,11] stressed that lamin B expression is constitutive for all cells, while lamine A is additionally acquired only upon differentiation. Since in chicken lamin B2 seems to reflect the more dominant B form of the early embryo [9], we expect that monoclonal antibodies able to distinguish murine lamins B1 and B2 will provide a similar picture in murine embryogenesis.

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