Genomic structure of the mouse A-type lamin gene locus encoding somatic and germ cell-specific lamins

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Abstract Mouse A-type lamin genes were isolated. Structural analyses revealed that all the three known mouse A-type lamins (A, C and C2) were coded in a single genomic locus in a 22 kilobase DNA segment. The three lamins were coded in 12, 10 and 10 exons for A, C and C2, respectively, and shared 8 exons among them. Primer extension analyses identified possible transcription start sites for both A/C and C2 genes suggesting that the locus is under the control of two separate promoters, that is a somatic cell-acting promoter (for A and C) and a testis-specific promoter (for C2) which resides in the first intron of the A/C gene. Sequence characteristics of the possible promoter regions are discussed. Divergence of the two somatic cell-type lamins (A and C) is formally accounted for by differential selection of poly(A) sites together with lamin A-specific splicing.

Key words: Nuclear envelope; Lamin; Nucleotide sequence; Gene structure; Promoter; Differential processing

1. Introduction

Lamins are a group of proteins which constitute the nuclear lamina structure inside the nuclear membrane and are classified as members of the intermediate filament protein family ([1,2]; for review see [3–6]). In vertebrates two types of lamins, A-type and B-type, are known and each type of lamin contains multiple numbers of species. In mammals, two B-type lamins (B1 and B2 [7]) are constitutively expressed in all kinds of cells. On the other hand, A-type lamin (A and C) expression is developmentally regulated [8,9]. In addition, for both types, germline cell-specific lamins (B3 [10] and C2 [11]) have also been reported. Thus far in mammals lamin gene structures have been analysed only for B2 in mouse [12], and A and C in human [13]. Although genomic DNA has not been analysed, it is speculated that mouse B3 is coded in the same genomic locus as the B2 gene and expressed by differential splicing and alternative polyadenylation [10]. A and C are coded in a single genomic locus and thought to be expressed by differential processing of a primary transcript which is most probably transcribed from a single promoter [1,2,13]. It is also speculated that the C2 gene is coded in the A/C gene locus in mouse and expressed by differential splicing, because of its sequence similarity to lamin C [11].

In general, lamin function is thought to be mainly structural. Although detailed functions are still not well understood, interesting observations about lamin functions are accumulating ([14–18]; for review see [19]). Because A-type lamins are not expressed or are diminished in cells in early stages of embryogenesis, embryonal carcinoma cells and certain kinds of malignant cells [8,9,20–23], some relationship between the function of this type of lamin and the differentiated state of cells is speculated. An experiment to get insight into this possibility was done by Peter and Nigg [24]. They examined the effect of ectopically expressed lamin A in embryonal carcinoma cells, and they found no significant effect on cell morphology or in the cell's ability to differentiate.

As a step toward the analyses of developmentally controlled expression of A-type lamins, and to clarify the genetic structure of mouse A-type lamin genes, we have cloned and analysed mouse A-type lamin genes. In this report we describe the structural organization of the mouse A-type lamin gene locus and the possible promoter sequences and we show that the A-type lamin locus codes not only somatic cell-type lamins (A and C), but also testis-specific lamin C2, which is probably transcribed from a promoter separate from the somatic cell-type lamins' promoter.

2. Materials and methods

2.1. Library construction and screening

A mouse genomic library cloned into the lambda-DASH vector (Stratagene) *Bam*HI site was constructed from size-fractionated (15–20 kb) *Sau*IIIA partial digests of mouse embryonal carcinoma cell (MC12, [25]) DNA according to the method described in [26]. The library (1.5×10^6 pfu) was screened using cloned lamin A and lamin C cDNAs [27] as probes. Hybridization probe was labelled with ^{32}P by the random priming method [26] and plaque hybridization was carried out using Hybond-N+ membrane (Amersham) according to the manufacturer's instructions. Positive plaques were purified through two additional cycles of plating and hybridization steps, and DNA was then extracted from liquid phage lysate [26].

2.2. Exon mapping and sequencing

The genomic restriction map was made by aligning the common restriction fragments of lambda clone digests produced by *Bam*HI, *Eco*RI, *Xba*I and some other restriction enzymes and confirmed by genomic Southern analysis using MC12 DNA and lamin A and C cDNAs as probes. For C2 exon mapping, an oligonucleotide synthesized according to the published sequence (see section 2.3) was used as a probe. mRNA coding sequences for lamin A and C were mapped on the genomic map by Southern analysis [28] utilizing various restriction fragments of lamin A and C cDNA clones as probes. Exon-containing fragments (shown in Fig. 1a) were subcloned in Bluescript II vector (Stratagene), and sets of serial deletion clones for sequencing were made by the method described in [29]. DNA sequencing was performed using

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The nucleotide sequence data reported in this paper will appear in the GSDB, DDBJ, EMBL and NCBI nucleotide sequence databases with the following accession numbers: D49732, D49733, D49734, D49735 and D49736.

SSDI 0014-5793(95)00453-X

Sequenase Ver.2 (United States Biochemical) on both strands of DNA for all the sequences listed in this report.

2.3. Primer extension analyses

Primer extension analyses was done according to the procedure described in [26] including 50 μg/ml of actinomycin D in the reverse transcription reaction. 50 μg of total RNA from mouse cell line FM3A for the A/C gene, and 75 μg of total RNA from mouse testis for the C2 gene were used for their respective reactions. DNA primers with the sequences complementary to the regions marked in Fig. 2 were synthesized: for the A/C gene, 30mer at position −106 to −135; for the C2 gene, 17mer at position +14 to −3 were used. Both primers were purified by 20% acrylamide gel electrophoresis [26].

2.4. Other methods

Northern analyses were done according to the method described in [26] using Hybond-N+ membrane. RNA samples were prepared by the guanidinium thiocyanate extraction and CsCl centrifugation method [26]. Computer search for the transcription factor recognition sequences was performed using the Genetyx version 9.0 program (Software Development Co. Ltd.).

3. Results

3.1. Isolation and sequencing of mouse lamin A and lamin C genes

A mouse genomic library was constructed and screened using mouse lamin C and partial lamin A cDNAs [27] as probes. A total of seven independent positive clones were isolated and they were further analysed. Restriction analyses showed that each clone overlapped with at least two of the other clones and the clones covered about 45 kilobases (kb) of continuous genomic DNA region all together (Fig. 1a). The deduced genomic map (Fig. 1a) was confirmed by genomic Southern analyses (data not shown). By Southern analyses using various portions of lamin A and C cDNAs as probes, complete cDNA coding regions for both lamins were found to be contained within these clones (data not shown), and the coding regions were localized on the genomic map. Appropriate exon-containing restriction fragments were subcloned (see Fig. 1a); then the sequences of all the exons, exon–intron junctions and relatively short introns were determined. As shown in Figs. 1a and 2, lamin A and lamin C cDNA sequences were found to be coded in 12 and 10 exons, respectively, within a 22 kb segment of genomic DNA. Exons 1 through 9, encoding most of the lamin C protein (except for 38 out of 574 amino acids), were completely shared by both lamins. Lamin C exon 10, which was 207 bases long and the last exon for lamin C, was partially shared by lamin A: the lamin A exon 10 comprised the first 96 bases of the lamin C exon 10. Lamin A specific splicing at this position eliminated a poly(A) site used in lamin C, then resulted in acquisition of two lamin A-specific exons, exon 11 and exon 12. Though exon 12 was the longest exon (951 bases), it coded only eight amino acids (including a C-terminal CaaX motif [30]), all of which are eliminated during the processing of prelamin A [31].

Thus mouse lamin A and lamin C are coded in a single genomic region, as had been suggested in mouse [27] and shown in humans [13]. The exon sequences were identical to the published cDNA sequences in both lamin A [27] and lamin C [32] except for the sequences marked in bold letters in Fig. 2: they include nine positions in exon 1, one in the 3′-noncoding sequence of lamin C exon 10, two in lamin A exon 11, and three in the 3′-noncoding sequence of exon 12. The base substitutions which are involved in coding sequences are found in 6 codons over all. Among them, three which cause amino acid changes may be worth mentioning here: two in exon 1 and one in exon 11. In the former case, the cDNA sequence TCT (reported in [32]) is changed to CTC in the genomic sequence, thereby the amino acid sequence in codons No. 118 and 119, VC, is changed to AR. In the latter case, the cDNA sequence GCC (reported in [27]) is changed to genomic sequence GTC at codon No. 623, thereby the amino acid is changed from A to V. In both cases the sequence changes resulted in the mouse genomic sequence perfectly matching the human [1,2] and rat [33] lamin cDNA sequences in the corresponding regions. In line with this, a mouse lamin C cDNA clone which has an identical sequence to the genomic sequence including all the above 9 positions and is 30 bases longer at the 5′-end than the published sequence [32] has been isolated in our laboratory [27] (sequence data has not been published). As for the splicing sites, all the introns had sequences matching the splicing junction site consensus 'GT-AG' sequences and had pyrimidine-rich stretches at the 3′-end of the introns (see Fig. 2). The first intron was exceptionally long (about 14 kb) among the introns of these genes.

3.2. Identification of an exon unique to lamin C2

Recently, Furukawa et al. [11] reported a new germline cell-specific lamin mRNA species in mouse, which they named lamin C2 from its strong sequence similarity to lamin C. The first 71 bases of C2 cDNA differed from the lamin C sequence, but thereafter the C2 cDNA sequence was identical with the lamin C sequence, and Furukawa et al. suggested that the lamin C2 mRNA is generated from the somatic A-type lamin gene by differential splicing during spermatogenesis. To examine this notion we searched for the exon sequence unique to the lamin C2 5′-end. Southern analysis with an oligonucleotide probe made from the published C2 sequence (see section 2 and Fig. 2) revealed that the C2-specific sequence was contained in lambda clone A3–2 (data not shown). After subcloning and mapping, the sequence spanning the C2-specific exon was determined. As shown in Fig. 2, a sequence identical to the published C2-specific sequence, C2 exon 1, was identified. The C2 exon 1 resided in the first intron of the A/C gene at about 4 kb upstream of A/C-exon 2 and was followed by an intron that commenced with consensus GT dinucleotides whose excision results in connecting the C2 exon 1 to A/C exon 2.

Thus, it was clarified that the mouse A-type lamin gene locus codes not only lamins A and C but also C2, which thereby share 8 exons among them. Comparison of exon composition of individual A-type lamins is illustrated in Fig. 1b.

3.3. Primer extension analyses of lamin A/C and C2 transcripts

Although it has been suggested that lamin C2 is generated from the somatic A-type lamin gene by differential splicing, and the above described results support it, it is not known whether A/C and C2 are coded in transcripts which are transcribed from the same promoter or not. To clarify the transcription start sites for lamin A/C and C2 transcripts, primer extension experiments were carried out. A/C transcripts were analyzed using a single primer because, although no direct data were available about whether the lamin A and C genes are transcribed from the same transcription start site, we assumed they are, since they seemed to share the same exon 1 sequence [1,2,13]. As shown in Fig. 3a, a major extension band was seen at position −203 accompa-

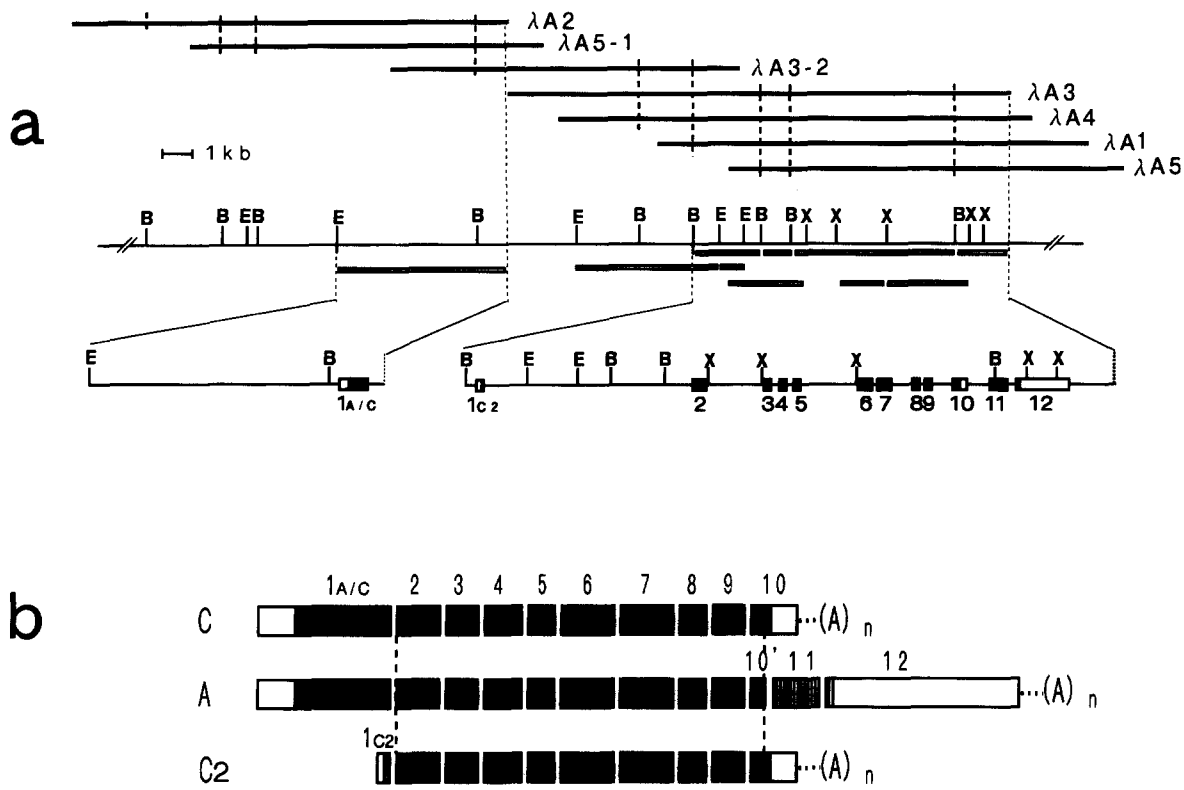

Fig. 1. Structure of mouse A-type lamin gene locus. (a) Alignment of λ-DASH clones (top horizontal bars) and deduced genomic restriction map (with expansions at the bottom) are shown. Symbols B, E and X denote restriction sites for *Bam*HI, *Eco*RI and *Xba*I, respectively. Dotted vertical lines crossing the lambda clone bars mark either *Bam*HI site alignment (thick lines) or end points of λ clones used for subcloning (thin lines). Key subclones used for sequence analyses are shown by open bars at the corresponding positions below the genomic restriction map. Boxes in the expanded genomic maps indicate the sizes and positions of the exons (closed box, coding region; open box, noncoding region). (b) Comparison of the three A-type lamin mRNA structures is shown in diagrams. Boxes represent exon components of each lamin sub-species, common exons are aligned. Regions between the dotted vertical bars are common in all three lamin species. White regions denote non-coding regions. Hatched regions are coding sequences unique to a single lamin. Exons 11, 12 and exon 1c2 are unique to lamin A and lamin C2, respectively. Exon 10′ is common to the 5′ region of exon 10 (see text).

nied by nearby minor bands (position '1' designates the first base of the initiation codons for lamin A and C proteins; Fig. 2). Less strong extension bands were also seen at around −241. Transcription initiated at these sites will give about 3.1 kb and 2 kb long mRNAs for lamin A and lamin C, respectively, and these sizes are compatible with the mRNA sizes measured by Northern analyses (data not shown). Computer search for *cis*-elements within a few hundred bases upstream of the start sites found various recognition sequences for known transcription factors, e.g. PEA1 site [34] at −211, and SP1 site[35] at −301 (see Fig. 2 and section 4). Although no typical 'TATA' sequence was found in this region, the closely related sequence 'TATTA' was seen at −236, which was about 30 bases upstream of the major putative transcription initiation site.

In the lamin C2 gene, as shown in Fig. 3b, a major extension band corresponding to position −40 was detected (position '1' designates the first base of the initiation codon for lamin C2 protein; Fig. 2). Less strong bands were also seen at positions −45 and −52. A computer search for *cis*-elements upstream of the possible initiation sites revealed various recognition sequences of known transcription factors, such as SOX-5 [36], Tet-1 [37] and TAP-1 [38] binding sequences, which have been assigned as important for the expression of testis-specific genes (see Fig. 2 and section 4).

4. Discussion

4.1. Organization of mouse A-type lamin genes

In this study we showed that mouse lamin A and lamin C are coded in a single genomic locus. In addition we have identified the germline-specific A-type lamin, lamin C2 [11], sequence in this region, clarifying that all the mouse A-type lamins are coded in a single genomic locus. The three A-type lamins share 8 exons out of 10 (for C and C2) or 12 (for A) exons among them. The genomic structure and primer extension analyses indicated that the expression of A-type lamins was under the control of two separate promoters, i.e. a somatic cell-acting promoter for A and C, and a germ cell-acting promoter, which resides in the first intron of the A/C gene, for lamin C2. Although we cannot eliminate the slight possibility that lamin C2 message is transcribed from the same promoter as somatic cell-type lamin and generated by differential splicing (in this case there has to be an additional as yet undetected very short exon at the 5′-end of the C2 message), we think it is unlikely. Divergence of the two somatic cell-type lamins can be formally accounted for by differential selection of poly(A) sites together with lamin A-specific splicing. The precise mechanism for the differential processing of 3′-sequences in the somatic lamin transcripts is not clear, but, because the message started from

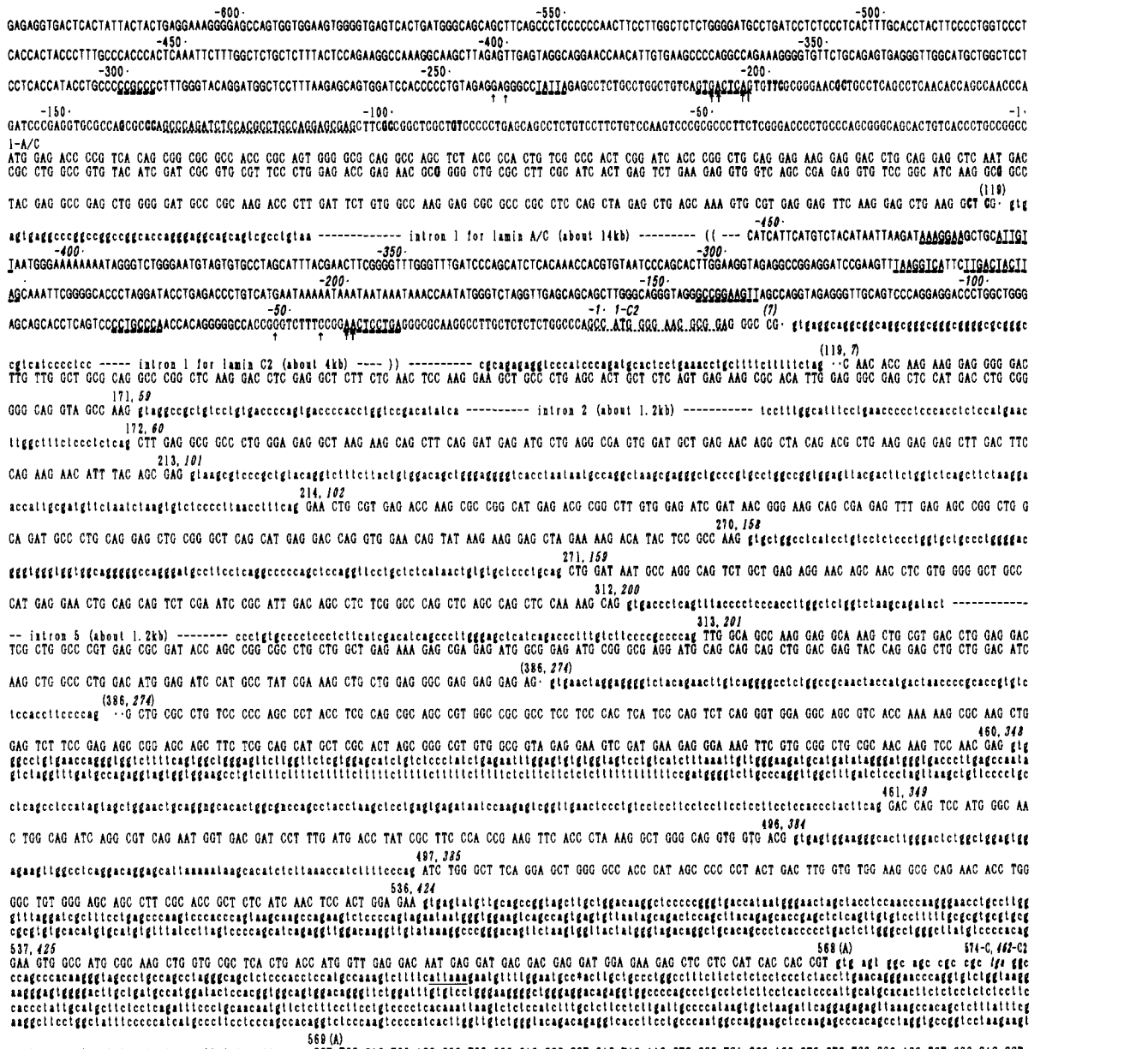

Fig. 2. DNA sequence of mouse A-type lamin gene locus. Genomic DNA sequence codes for lamin A, C and C2 are listed. For both A/C and C2 genes the first base of the initiation codon is designated '1' (for the C2 gene italics are used). Sequences upstream from the 5′-ends of the reported cDNA sequences [32] are typed in gothic. Protein coding sequences are divided into codons and the amino acid numbers (in italics for C2) are put at the beginning and ending codons of each exon (numbers in parentheses are the amino acid numbers whose codons are split by introns). Intron sequences are typed in lower case. Except for intron 1 (for A/C and C2), intron 2 and intron 3, complete intron sequences are listed. C2 exon 1 (in parentheses) resides within the first intron for the A/C gene and thereafter the C2 gene completely overlaps with the C gene. The sequences homologous to the AATAAA consensus poly(A) signal are marked by underlinings. Where the sequence is different from previously reported sequences (those in A/C exon 1 and C exon 10, see [32]; A exon 12, see [27]) it is typed in bold letters. Mapped mRNA start sites are indicated by arrows. Possible promoter element sequences (see text) are marked by dashed underlines. Primer sequences used (see section 2) are marked by dotted underlinings.

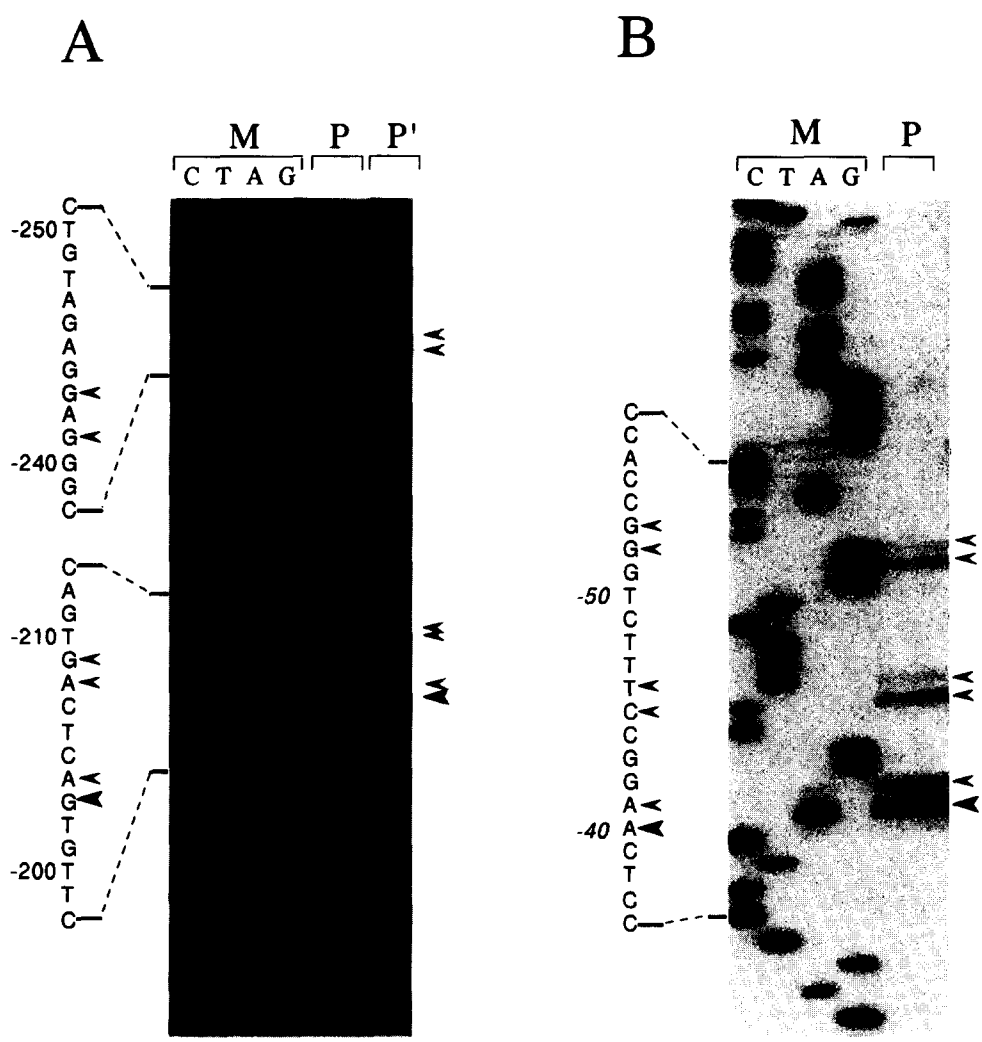

Fig. 3. Primer extension analyses of A/C and C2 transcripts. The gel analyses of the primer extension products together with marker sequence ladders are shown. (A) Analysis of lamin A/C gene transcripts. (B) Analysis of lamin C2 gene transcripts. M denotes the lanes of marker sequence ladders and P denotes the lanes (two lanes with slightly different amounts of samples) of primer extension products. The lane P' is the same as lane P but with longer exposure. The positions of primer extension bands are marked by arrowheads. Sequencing for markers was done by the dideoxy chain termination method using the same 5'-end labelled primers used for primer extension reactions. For convenience the sequences of the template strands are listed instead of the actual sequence read from the gel.

the lamin C2 promoter seems to have only lamin C-type 3'-sequence [11], it is likely that the formation of different 3'-ends is a regulated process.

Lin and Worman [13] have reported the human lamin A/C gene structure. Although the presence of the extra exon for C2 lamin has not been examined in humans so far, otherwise the genomic structure of the A-type lamin gene locus in mouse and humans is very similar. In both species the positions of intron insertion were exactly conserved, and also the length of corresponding introns were very similar, including the exceptionally long length of the first intron, in which the first exon for the C2 gene resides in mouse. Accordingly, conservation of the general exon/intron-organization between the mouse A-type lamin gene and other intermediate filament protein family genes, including the mouse lamin B2 gene [12], was evident, as has also been discussed for the human A-type lamin gene structure [13]. Recently the mouse lamin B1 gene structure was also analysed in our laboratory (Maeno et al., manuscript in preparation), and more detailed comparison of genomic structures among all the mouse lamin genes will be described in that manuscript.

4.2. *Structure of the putative promoter regions and implications for their functions*

In contrast to B-type lamins, A-type lamin expression is developmentally regulated: lamin A and lamin C are devoid in cells in early stages of embryogenesis [8,9], and lamin C2 is specifically expressed in pachitene-stage spermatocytes [11]. Primer extension analyses identified possible transcription start sites for both A/C and C2 genes, giving some information about presumptive promoter structures.

The major mRNA start site mapped for the A/C gene (position −203) roughly corresponded to the published lamin C cDNA 5'-end [32], and there is an another lamin C cDNA clone whose 5'-end is closely mapped to the upstream minor start sites ([27]; see section 3, and Figs. 2 and 3). In our ongoing in vitro transcription experiments, a run-off product whose 5'-end mapped to the major start site mentioned above was detected

(Nakajima, unpublished result). Thus we concluded that the region around the start sites mapped here has the promoter function for lamin A/C gene expression. Among the possible transcription factor recognition-sequences seen in this region, the SP1-binding site (at −301) and PEA1-binding site (at −211) are worth mentioning briefly. In a preliminary in vitro transcription experiment the transcription efficiency was markedly reduced by deleting the upstream sequences that include the SP1 site (Nakajima, unpublished result). PEA1 (the mouse equivalent of AP1) is known to be deficient in embryonal carcinoma cells, and activated during differentiation of the cells [34]. Kaufmann et al. [23] reported an activation of lamin A/C expression by v-ras^H in human lung cancer cells and suggested a possible implication of AP1 for lamin A/C expression. These observations, though they certainly do not prove, may suggest possible involvement of the PEA1 site in the developmentally controlled expression of the lamin A/C gene. In order to clarify the mechanism involved in the regulated expression, functional analyses of the promoter region of the A/C gene in vivo and in vitro are in progress in our laboratory.

Lamin C2 is thought to be an A-type lamin which is specifically expressed in spermatocytes [11]. This protein is of interest with regard to its testis-specific gene expression as well as its function. Primer extension analysis identified closely scattered multiple possible transcription start sites. From the estimated C2 message size by Northern blot analyses (about 1.6 kb; data not shown, and [11]) it is possible that the mapped start sites are part of bona fide transcription start sites, although all the sites resided slightly 3′- of the 5′-end of the reported lamin C2 cDNA clone [11]. Within some 120 bases immediately upstream of the mapped sites no classical TATA-box was found, though an AT-rich sequence, which is contiguous to a mouse B1 repetitive sequence [39], was located just 5′ of this region. Upstream of the mapped start sites various sequences that might be relevant to the spermatocyte-specific promoter were seen. Sox-5 is a member of the SRY protein family and known to be specifically expressed in testis [36]. A Sox-5 binding sequence, AACAAT, is found in the inverted orientation at −410. Ets family proteins are transcription factors which share the 'GGAA' core consensus sequence for their binding. There are two possible Ets binding sites at −428, AAAGGAA, (TAP-1 binding consensus sequence) which is reported to be important for pachytene spermatocyte-specific expression of the mouse Pgk-2 gene [38], and at −145, GCCGGAAGTT (Ets-1 binding sequence [40]). The tACE-I sequence, TGAGGTCA, is reported to be a critical sequence for the expression of the testis-specific form of angiotensin-converting enzyme [41]. A homologous sequence, TAAGGTCA, is located at −273. Lastly, some sequences homologous to the promoter region of the mouse protamin 1 (Prm-1) gene (a well-characterized gene specifically expressed in testis) are also found: CCTGCCCA (termed Box B [42]) and TGACTTCATAA (Tet-1 binding sequence [37]) are reported to play roles in spermatide-specific expression and an exact Box B sequence and a sequence homologous to the Tet-1 binding sequence, TGACTACTTAG, are located at −77 and −261, respectively. In addition, an identical nonamer, AACTCCTGA, is seen at −41 of the C2 gene and just upstream of Box B in the Prm-1 gene in [42]. These features may suggest that the region upstream of the mapped initiation sites has promoter function for the testis-specific expression of the C2 gene. Analysis to identify the functional elements is planned.

As yet, lamin functions are not well understood. It seems that A-type lamins are not essential at least for the proliferation of certain types of cells such as cells in early stages of embryogenesis and embryonal carcinoma cells, but they become expressed as cell differentiation proceeds. Although this report does not deal with lamin functions, we hope that the data presented will give bases for the future study of functional aspects of A-type lamins, such as by means of transgenic and gene disruption experiments.

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