The Mouse Ribosomal Protein L7 Gene

ITS PRIMARY STRUCTURE AND FUNCTIONAL ANALYSIS OF THE PROMOTER REGION*

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The expressed gene coding for mouse ribosomal protein L7 (rpL7) was structurally and functionally characterized. It consists of seven exons, spans 3107 base pairs, and its coding sequence initiates within exon 1. The primary structure of mouse rpL7 (270 amino acids), as inferred from the nucleotide sequence of the exons of the gene, and from the cDNA, is 12 residues longer than the rat counterpart. The rpL7 gene shares common structural features with most other mammalian ribosomal protein genes analyzed thus far. These include the lack of a canonical TATA box and a major transcription initiation site at a cytidine residue embedded in a stretch of 14 pyrimidines, flanked by C + G-rich regions. Transient expression assays revealed that the promoter region of rpL7 gene bears several regulatory elements, both upstream to the cap site and within the transcribed portion of the gene. One internal regulatory element was assigned to the first intron and a second one to a 20-base pair region spanning the first exon-intron junction. The activity of a deletion mutant of rpL32 gene, lacking its internal elements, can be rescued by insertion, in the sense orientation, of the corresponding elements from the rpL7 gene. The unique spatial organization of the regulatory elements in rpL7 gene, as well as in other murine ribosomal protein genes examined thus far, might indicate that this common architecture is involved in the mechanism coordinating their expression.

The biosynthesis of mammalian ribosomes requires an equimolar accumulation of four RNA molecules and over 70 different ribosomal protein (rp) species. This stoichiometry is achieved by coordinate regulation at various levels of gene expression and under diverse physiological conditions (for review see Ref. 1). Thus, the steady state levels of different rp mRNAs in proliferating murine cells is relatively uniform (2) resulting from comparable transcriptional efficiencies (3). During differentiation of mouse myoblasts, the transcription of rp genes is coordinately repressed (4). In contrast, the abundance of various rp mRNAs is elevated in regenerating rat liver (5). Translation of rp mRNA is also coordinately controlled, as was shown in mouse lymphosarcoma cells upon glucocorticoid treatment (6) and in differentiating (4) or insulin-treated mouse myoblasts (7).

A comparative analysis of the promoter region of three mouse rp genes (8–11), and to a lesser extent of two different human rp genes (12, 13), has revealed several common features including (a) the lack of a canonical TATA box, and (b) the major site of transcriptional initiation is located at a cytidine residue which is embedded in a pyrimidine tract, flanked by sequences of high G + C content. More refined characterization of the elements comprising the promoters of mouse rpL30, rpL32, and rpS16 genes disclosed a similar general organization, particularly the presence elements downstream of the cap site (3, 14–17). Yet the trans-acting factors interacting with the cis-regulatory elements of rpL30 and rpL32 are clearly distinct from those interacting with the corresponding elements in rpS16. Moreover, the latter lacks transcription regulatory elements downstream of exon 1 (3). To verify whether other rp genes can be categorized according to their promoter architecture into one of these types (L30/L32 or S16), or yet belong to a distinct type, it is essential to carry out a comparative study of the structure, organization, and regulatory properties of additional rp genes.

Screening of mouse genomic library resulted in the isolation of seven genes encoding for mouse rpL7, of which only one (L7-16b) exhibits a high degree of homology with L7 mRNA and contains introns, whereas the others appear to be processed pseudogenes (18).

In this report we present a detailed structural and functional analyses of the transcriptionally active mouse rpL7 gene (L7-16b). Our data indicate that this gene is 3.1 kilobase pairs long and encodes for a protein of 270 amino acids which is 12 residues longer than the rat counterpart. The rpL7 gene is composed of seven exons, exhibits distinctive structural features unique for rp genes and requires sequences within exon 1 and intron 1 for its expression. The intragenic regulatory elements coevolved in several mammalian rp genes (3, 14–17) and might be involved in the coordination of rp gene expression.

EXPERIMENTAL PROCEDURES2

RESULTS

Primary Structure of rpL7 Gene—The isolation of an intron-containing gene (L7-16b) and its preliminary restriction

*This research was supported by grant 949-0070/2 from the United States-Israel Binational Science Foundation (BSF), Jerusalem, Israel and by the Sir Zelman Cowen University Fund. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1794 solely to indicate this fact.

2 Portions of this paper (including "Experimental Procedures" and Figs. 2 and 3) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.
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The primary structure of mouse rpL7 gene. **a**, restriction map and sequencing strategy for rpL7 gene. **R**, EcoRI; **H**, HaeIII; **N**, NcoI; **B**, BamHI; **P**, HpaII; **G**, BglII; **K**, KpnI, **F**, HindIII. Only the enzyme sites used for end labeling are shown. Each arrow designates the direction and the extent of nucleotide sequence that was determined. 50% of the sequence was determined from both strands. **b**, nucleotide sequence of mouse rpL7 gene. Flanking and intervening sequences are displayed in lowercase letters and exons in uppercase letters. The L7 protein sequence is indicated under the sequence of the DNA. The cap site as determined by primer extension (Fig. 2) is numbered 1. The polyadenylation signal (underlined) is located 20 nucleotides from the end of the gene, as established by colinearity with the 3′ end sequence of L7 processed gene (Fig. 3). Exon–intron boundaries were recognized by alignment of sequences of the rpL7 gene and those of the L7 mRNA (cDNA) and of the L7 processed gene (Figs. 3 and 4).

Fig. 1. The primary structure of mouse rpL7 gene. **a**, restriction map and sequencing strategy for rpL7 gene. R, EcoRI; H, HaeIII; N, NcoI; B, BamHI; P, HpaII; G, BglII; K, KpnI, F, HindIII. Only the enzyme sites used for end labeling are shown. Each arrow designates the direction and the extent of nucleotide sequence that was determined. 50% of the sequence was determined from both strands. b, nucleotide sequence of mouse rpL7 gene. Flanking and intervening sequences are displayed in lowercase letters and exons in uppercase letters. The L7 protein sequence is indicated under the sequence of the DNA. The cap site as determined by primer extension (Fig. 2) is numbered 1. The polyadenylation signal (underlined) is located 20 nucleotides from the end of the gene, as established by colinearity with the 3′ end sequence of L7 processed gene (Fig. 3). Exon–intron boundaries were recognized by alignment of sequences of the rpL7 gene and those of the L7 mRNA (cDNA) and of the L7 processed gene (Figs. 3 and 4).
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**Fig. 4. The primary structure of L7 mRNA.** a, restriction enzyme map and sequence strategy of mouse L7 cDNA. D, Ddel; R, EcoRI; G, BglI; K, KpnI. b, nucleotide sequence of mRNA (cDNA) from position 757 to position 837) was derived from exon 6. The rat L7 cDNA and protein included between the triangles (V) (spanning positions 30-757) was sequences were published previously (38). Matching amino acids are determined from the cloned cDNA. The sequence of the first 30 nucleotides was directly determined from sequencing of the extended primer (Fig. 2) and from exon 1 (Fig. 1). The 3' end of the coding sequence of mouse L7 mRNA (cDNA) from position 837 to position 877 was derived from exon 6. The rat L7 cDNA and protein sequences were published previously (38). Matching amino acids are indicated by dashes matching nucleotides are shown by asterisks. Missing codons and amino acids within the rat sequences appear as dots and as spaces, respectively.
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Fig. 5. Expression of the rpL7 gene as determined by Northern blot analysis. DNA replicate samples (10 μg) of EcoRI-digested mouse liver genomic DNA were size-fractionated in 0.7% agarose gel, blotted onto Nytran filter, and hybridized with nick-translated L7 intron probe (a) and L7 cDNA probe (b). The single 1.55-kb band demonstrates the uniqueness of the intron probe. RNA replicate samples (5 μg) of poly(A)+ nuclear RNA from mouse P1798 lymphosarcoma cells were electrophoresed in a denaturing 1% agarose gel. The RNA was transferred to Nytran filter and hybridized with probes a and b. The sizes of the RNA components were determined with appropriate size markers (ranging from 1.4 to 9.5 kb obtained from Bethesda Research Laboratories). The cDNA probe revealed all components (including those which are barely detected from Bethesda Research Laboratories) of the RNA components, including those which are barely detected from Bethesda Research Laboratories. Taking into account the size of the gene (3107 bp) and the assumption that the poly(A) tail on the pre-mRNA is 200 nucleotides long (43), the apparent discrepancy between the number of introns in rpL7 gene (six) and the number of RNA components revealed by the cDNA probe (ten) can be explained by the presence of several alternative intron excision pathways, as was shown previously for other rp pre-mRNAs (8, 9). Nevertheless, unless additional intron-specific probes are used, we cannot exclude the possibility that the cDNA probe also detects processing intermediates of another intron-containing gene(s).

Fig. 6. The effect of various L7 promoter segments on CAT activity. Mouse Ltk− cells were transfected with various L7-CAT constructs incubated for 40 h, harvested, and CAT assays were performed on the cell extracts. In the schematic representation of the L7-CAT constructs, the 5′-flanking sequence is denoted as a thin line, exon 1 as a box, and intron 1 sequences as a thick solid line. A dotted box and a dotted thick line represent mouse c-myc exon 1 and intron 1 sequences. The average CAT activity (of at least three separate transfection experiments with results differing by no more than 20%), corrected for variation in transfection efficiencies as described under “Experimental Procedures,” and normalized for pL7CAT3, is listed at the right.
of this motif in transcriptional regulation has been demonstrated by base substitution within the δ-factor binding site, of rpL30 which led to a decreased binding of the δ-factor, with concomitantly reduced promoter activity (3). To examine whether this or other sequences in its close vicinity might be involved in transcriptional regulation of rpL7, we have introduced them inversely within a 20-bp fragment (+24 to +46), downstream to the deleted L7 promoter (pL7as-L7CAT4, Fig. 6). Clearly, despite the opposite orientation of this sequence it exhibits a two fold stimulatory effect on the CAT activity. This stimulation might reflect a component of the internal control element which is orientation independent and therefore should function at the DNA level.

Since the mouse c-myc gene has a positive transcriptional element at the 3' end of exon 1 (65), a region that also contains a binding site for the rpL30/rpL32 δ-factor (14), we have examined its ability to complement the L7 deletion mutant. A 59-bp fragment (+521 to 579), spanning the exon 1-intron I boundary of c-myc gene and containing the motif CAGCCTTC which is similar to that found in the δ-factor binding sites of rpL30 and rpL32, was cloned downstream to the rpL7 deleted promoter (pmyc-L7CAT4). Fig. 6 shows that the myc transcriptional regulatory element causes about 4-fold induction in the CAT activity, quantitatively resembling its effect on the activity of the enhancerless SV40 early promoter (65).

In most constructs the first AUG codons is the L7 initiation codon at +25 immediately followed by a TAA termination codon at +40 except for pL7CAT4 and pL7CAT7 where the stop codon is located in the 5'-untranslated region (UTR) of the CAT transcript. Differences in CAT activity can, theoretically, be achieved by interference at any possible level during gene expression. Thus, the abrupt drop in CAT activity, achieved by deleting the intragenic sequences from +248 to +27, could be attributed to impaired translation of the transcript with the shorter 5' UTR, rather than decreased abundance of the respective mRNA. To address this question we stably transfected mouse L cells with pL7CAT3 (~300 to +248) and pL7CAT4 (~300 to +27) and evaluated the relative abundance of the CAT DNA sequences and the respective transcripts (Fig. 7). Northern blot analysis revealed that the CAT transcript in cells transfected with pL7CAT3 was readily observed as compared to the barely detectable CAT transcript (at least 100-fold lower) in cells transfected with pL7CAT4 (Fig. 7a). Equal amounts of RNA from both transfected cultures were loaded on the gel, as evident by the similar abundance of the endogenous L7 mRNA detected in the parallel pair of lanes in the same blot (Fig. 7b). It is worth noting that the ratio of CAT activity in these transfected cells paralleled that of the abundance of the respective mRNA (data not shown). A Southern blot of genomic DNAs from the respective cultures was hybridized with a probe containing CAT sequences as well as unique sequences from intron 3 of mouse rpL32, serving as an internal quantitative reference (Fig. 7c). Densitometric scanning of the autoradiogram revealed six copies of pL7CAT3 per cell as compared with one to two copies of pL7CAT4. These results clearly suggest that deletion of intragenic sequences (+27 to +248) exerts its repressory effect on CAT expression by diminishing the abundance of CAT mRNA.

Internal Regulatory Elements of rpL7 Gene Can Rescue an rpL32 Deletion Mutant—Having demonstrated that the spatial organization of regulatory elements, in the 5'-flanking and internal sequences of the rpL7 promoter region, is similar to those of rpL30 and rpL32, it was interesting to verify whether the internal elements are also functionally equivalent. It has been shown previously that deletion of internal control elements (ICEs) of the rpL32 promoter results in a substantial decrease in transcriptional activity (13, 14). An even greater repressing effect is apparent upon deletion of internal sequences (from +27 to +248) of the L7 promoter (compare pL7CAT3 and pL7CAT4 with pL32CAT5 and pL32CAT9 in Fig. 8). When the L7 ICE was inserted within a 225-bp fragment (+24 to +248) or a 97-bp fragment (+24 to +120) in the 5’ to 3’ orientation, immediately downstream of the L32 sequences in an L32 mutant, lacking its own ICE (+12 to +77), they substituted for the missing element (Fig. 8, pL7-L32CAT9a and pL7-L32CAT9c, respectively). However, the deletion mutant not only can not be rescued if these two L7 fragments were placed in the reverse orientation, but it was even further repressed (Fig. 8, pL7-L32CAT9b and pL7-L32CAT9f). It is noteworthy that replacement of L32 ICE by L7 ICE renders the hybrid promoter more active than the intact L32 promoter (compare pL32CAT3 with pL7-L32CAT9a and pL7-L32CAT9c, Fig. 8). These results are consistent with the apparent difference between L7 and L32 promoters (pL7CAT3 and pL32CAT5, Fig. 8). Interestingly, insertion in the sense orientation of a 23-bp segment (+24 to +46), which contains the dominant component in rpL7 ICE (Fig. 6), can only partially complement the L32 mutant (pL7-L32CAT9d, Fig. 8). However, unlike the two longer segments (225 and 97 bp), when placed in the antisense orientation, it still exerts a stimulatory effect (pL7-L32CAT9f, Fig. 8). It seems that the L7 and the L32 promoters are differentially affected by the L7 ICEs. Thus, the L7 promoter activity is mostly impaired by deletion of a 20bp segment (+28 to +46).
spanning the first exon-intron junction (compare pL7CAT8 and pL7CAT7 in Fig. 6), whereas that of the L32 mutant by a deletion of the downstream L7 intron sequences spanning positions +47 to +248 (compare pL7-L32CAT9s with pL7-L32CAT9d in Fig. 8).

**DISCUSSION**

**Evolution of rpL7 Gene** — A comparison of the amino acid sequence of mouse rp L7, as inferred from the nucleotide sequence, with that of the rat demonstrates differences in composition, as well as in size. The 258 amino acids of rat L7 exhibit 96% homology with the mouse counterpart as compared with 100% homology among mouse, rat, and human L32 (8, 44, 45) or S6 (46-48) and between mouse and rat L30 (9, 49) or human and Chinese hamster S14 (13, 50). However, even more striking is the occurrence of 10 extra residues (positions +27 to +46).

**Fig. 8.** CAT activity in L32-CAT deletion mutant, lacking sequences from exon 1 and intron 1, can be restored by the corresponding sequences from rpL7 gene. Mouse Ltk- cells were transfected with various CAT constructs driven by L7, L32, and L32-L7 hybrid promoter segments. After 44 h cells were harvested, and cell extracts were used for CAT assay. The rpL7 and rpL32 regions used as a promoter in each construct are schematically presented. The 5'-flanking is denoted as a thin line; black and dotted boxes represent exon 1 of L7 and L32 genes, respectively. Thick solid and dotted lines represent intron 1 of L7 and L32 genes, respectively. The average CAT activities in extracts of cells transfected with each construct were normalized to the value for pL7CAT3 and listed at the right. The data for each construct were obtained from three separate transfection experiments with results differing by no more than 25%. Variations in transfection efficiencies were controlled as described under “Experimental Procedures.”
internal regulatory elements exert their effect as typical enhancers (62, 64, 87, 88). Others, however, have been shown to function only in a position and orientation dependent manner (57, 88). The L7 ICE, with its two components, cannot be referred to as an enhancer-like element, since it functions only in its sense orientation. Likewise, analysis of the intron regulatory element of rpL32 revealed that its activity is also repressed by translocation or inversion (15). Nevertheless, the short L7 regulatory element (+24 to +46) seems to operate in an orientation independent manner, when linked to the L32 promoter.

The contribution of L7 ICE to the CAT expression clearly cannot be attributed to a nonspecific effect of the upstream AUG codon within the L7 sequence for the following reasons: (a) the L7 initiation codon is present both in pL7CAT3, which we have considered to include the fully active promoter and in pL7/CAT4 which exhibits only 1% of the activity of the former; (b) a similar stimulatory effect of the 23-bp segment, spanning positions +24 to +46, can be obtained when positioned in the sense or the anti-sense orientations, with respect to the L32 mutant promoter. This is evident despite the fact that the resulting transcript of the latter lacks the AUG codon; (c) removal of most of the intronic sequences (from position +46 to +248) leads to a marginal effect on the L7 promoter (compare pL7CAT8 with pL7CAT6, Fig. 6), but a very dramatic one on the L32 promoter (compare pL7-L32CAT9a with pL7-L32CAT9d, Fig. 8). This prominent discrepancy is apparent, although the upstream L7 initiation codon is similarly positioned with respect to the CAT sequence in both pL7CAT8 and pL7-L32CAT9d.

Northern blot analysis indicates that deletion of the L7 ICE from pL7CAT4 greatly impaired the accumulation of the CAT transcript (Fig. 7). Although we have not directly measured the relative rate of transcription, several lines of circumstantial evidence support the notion that these ICES function at the transcriptional level. (a) The requirement of the intron promoter element of rpL32 for maximal transcriptional activity was demonstrated by run-on assay in isolated nuclei (18). This element together with the rpL32 exon regulatory element can be efficiently substituted by the L7 ICE (+24 to +120). (b) A 23-bp segment, spanning the first exon-intron junction from rpL7 gene, contains a motif similar to that found in rpL30/rpL32 δ-factor binding site. This segment can exert a small, yet reproducible, stimulatory effect on both L7 and L32 promoter mutants, when placed in the anti-sense orientation. These results support the notion that an element within this sequence is orientation-independent and therefore likely to operate at the DNA level. (c) A 59-bp segment containing mouse c-myc transcriptional regulatory element, which also bears a similar motif, can partially complement the L7 promoter mutant. (d) The internal elements of rpL30 and rpL32 genes bind similar nuclear factors as evident by cross competition between these elements (3). Similarly, the L7 ICE specifically binds to a factor(s) in nuclear extracts and competes with the L30 ICE binding to nuclear factor(s).

In light of these facts it seems that the internal elements of rpL30 and rpL7 genes constitute a binding site(s) for some common factor(s), which conceivably involves with the transcriptional regulation of these genes. It is noteworthy that the rpS16 gene bears a distinctive set of cis-regulating elements, which do not cross compete with those of rpL30 and rpL32 (17). Yet, the ICES of the rpL32 gene can be adequately replaced by internal sequences (downstream to position +29) of the rpS16 gene (11). Thus, it seems that despite differences in the nature of the cis-regulating elements, the general architecture of the promoter modules of the rp genes can account for their coordinate expression.

Acknowledgments—We gratefully acknowledge the skillful technical assistance of Vladimir Ginzburg, Ester Hirschfeld, Yosv Morag, Nathalie Gluck, and Meir Cohen.

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Experimential Procedures

Sequencing. DNA was sequenced by a modified Maxam and Gilbert procedure or by the dideoxy chain-termination methods (4). In the latter case appropriate restriction fragments were used as single-stranded templates labeled with NTP or [α-32P]dNTPs or, alternatively, as double-stranded templates synthesized on T7 (3).

Primary ratification. A 4.7-kilobase (kb) EcoRI fragment of EcoRI-derived cDNA was labeled by nick translation and hybridized to a EcoRI digest to human ribosomal DNA as a probe. Productive hybridization was obtained from a 2.6-kb EcoRI fragment, and this fragment eventually was cloned into this EcoRI fragment, and this fragment eventually was cloned into the pBR322 plasmid vector. This primer was primed as described for 2.6-kb EcoRI fragments, and ligated to the HindIII and the blunt-ended EcoRI sites of pBR322, yielding plasmids G.

Isolation and purification. Nuclear acid RNA from Polyclonal extracts was isolated by the method essentially as described by Leder et al. (8). PolyA+ RNA was isolated as previously described (9). Nuclear RNA was isolated from tissue nuclei of 1- to 3-month-old animals by the method of Henegariu and Brownlee (10).

Culture and DNA Transfection. P153KLM mouse lymphoma cells were grown in suspension culture in RPMI 1640 medium (Gibco) containing 10% fetal bovine serum, 200 units/ml penicillin, 100 µg/ml streptomycin, and 2 µg/ml deaminoadenine (Gibco). DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of protein contamination by 5% phenol extraction. The DNA preparations were heated for 10 min at 37°C to remove single-stranded DNA, and the cells treated for 40 min at 37°C with medium containing 50 µg/ml 5-bromo-4-chloro-3-indolylphosphate (X-Gal). Cells were incubated for 24 hr before harvesting. Differences in transcription efficiencies were assessed by measurement of the amount of plasmid DNA extracted from cell nuclei, using the dot blot Northern and Southern blot analysis and hybridization for RNA transcription.

Mouse cells were stably transfected with DNAs of linearized cat expression plasmid (12). Cells and plasmids were ob (12). Cells and plasmids were obtained from manor coli. Preparation of plasmid for Southern blot analysis was performed according to Southern et al. (13). The percentage of transformation of G418-resistant cells was determined by colony assay. The DNA was extracted from tissue nuclei of 1- to 2-month-old animals and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates by the method of Hirt et al. (11) and was purified of DNA contamination by 5% phenol extraction. DNA was isolated from mouse organ homogenates}
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polymerase and cloned into the SalI site of pBluescript.

The plasmid pBlueL7 is a construct bearing one of the bacterial CAT sequence cloned into pBluescript, as described above. A 3.4 kb HindIII fragment containing mouse L7 gene was derived from pBlueL7, as described above. A 0.8 kb HindIII fragment containing bacterial CAT sequence was derived from pBluescript (CAT probe).

**Fig. 3** Determination of the 5' cap site by primer extension. Polyadenylated mouse fetal liver total RNA was used as template for primer extension analysis. The extended product (I) was analyzed by alkaline hydrolysis in a thermal cycler and oligonucleotide probe binding, followed by 7-deaza-guanosine synthesis, sequencing, and a size marker (II).

L7-18

3rd

---A---G---A-----

L7-18b

3844

---A---G---A-----

L7-18b

181

---A---G---A-----

L7-18b

3465

---A---G---A-----

L7-18b

241

---A---G---A-----

The nucleotide sequence of the 5' end of mouse L7 processed gene (L7-18). The first nucleotide corresponds to nucleotide 5' within exon 2 of the L7 gene (L7-18b). The sequence of exon 1 and the 5' flanking region, spanning nucleotide position 1 to 313, of rat L7 gene is aligned with the complementary sequence in L7-18. Matching nucleotides are shown by asterisks. Nucleotide numbers within the EL2 cDNA sequence correspond to the 5' end of the polyadenylation signal, which is underlined, indicates the 5' end of the L7 gene.
