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Importance of Introns for Expression of Mouse Ribosomal Protein Gene rpL32

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The importance of intronic sequences for expression of the mouse ribosomal protein gene rpL32 was evaluated by transfection experiments with a series of mutant constructs in which one or more of the three rpL32 introns was totally or partially deleted. When transiently transfected into monkey kidney (COS) cells or stably transfected into mouse L cells, a mutant that lacked all three introns was completely inactive. Constructs that contained intron 1, either alone or in combination with another intron, were expressed as efficiently as was the normal intact rpL32 gene. Constructs that lacked intron 1 but contained another spliceable intron, even one from a foreign gene, were expressed at about 10 to 20% of the maximum level. These results indicated that intron 1 contains an element that increases the level of expression by 5- to 10-fold. A comparison of internal deletion mutants localized the element to within the first 27 base pairs of intron 1. Nuclear run-on experiments with stably transfected COS cells demonstrated that this element functions at the transcriptional level. The element was inactive when translocated to a position upstream of the transcriptional start site or to a position within intron 3, which indicated that it does not have the properties of a typical enhancer. From these and other results, we conclude that introns have both a general and a specific role in rpL32 expression. The general role, which can be satisfied by any spliceable intron, is to ensure an efficient yield of RNA transcripts. The specific role is uniquely attributable to intron 1, which contains a transcriptional regulatory element near its 5′ end.

Since the discovery of introns over a decade ago, there has been considerable debate about whether they play a critical role in the expression of eucaryotic genes. With the elucidation of the organization and rules of expression of a vast number of genes, it has become clear that this question does not have a universal answer. Some genes carry transcriptional regulatory elements within their introns (3, 18, 21, 22). In other genes, introns are implicated in the quantitative regulation of mRNA production at the RNA-processing level (4, 9). In still others, they provide a means for producing multiple mRNA species from a single RNA transcript (5). Thus, in the evolution of each particular gene or gene family, intron sequences can apparently be coopted to perform a variety of functions.

In experiments designed to define the sequences responsible for regulating gene expression, it is often expedient to use minigene constructs which lack some or all of the introns or, alternatively, to link 5′-flanking regions and other isolated elements to a readily assayable reporter gene such as the gene encoding chloramphenicol acetyltransferase. Although these strategies have proven very powerful for localizing and characterizing transcriptional regulatory elements, they have the shortcoming that gene integrity is compromised and therefore any possible role of the missing portions of the gene cannot be properly evaluated. This shortcoming seems particularly relevant when one considers that genes evolve as integral units and not as isolated motifs.

There are certain properties of the mouse ribosomal protein gene rpL32 which are especially advantageous for examining whether intron sequences are important for expression. First, rpL32 is a relatively small and simple gene, spanning less than 3.5 kilobases (kb) and containing three relatively large introns (10). Second, there is an unmutated processed pseudogene of rpL32 (10), which provides a convenient source of intronless segments that can be substituted for selected portions of the normal gene. Third, only a short stretch of 5′-flanking sequence (less than 160 base pairs [bp]) is required for maximum rpL32 expression (1; R. Moura-Neto, K. P. Dudov, and R. P. Perry, Proc. Natl. Acad. Sci. USA, in press), which makes it easy to construct variant genes that contain all of the upstream regulatory sequences. In this study, we exploited these advantages to demonstrate that introns contribute to rpL32 expression in two distinct ways: specifically, by containing an important transcriptional regulatory element that may be part of the promoter, and generally, by increasing RNA transcript yield, presumably via an interaction with RNA-processing components.

MATERIALS AND METHODS

Plasmid construction. A 3.7-kb *Acc*I fragment of the expressed rpL32 gene cloned into pUC12 (11) and a subclone of the unmutated processed pseudogene rpL32-4A (10) served as the source material for all constructs. Using a selected restriction site in each of the four exons (Fig. 1), we exchanged appropriate segments of rpL32 and rpL32(ψ) by digestion and religation. All mutant constructs were inserted into pUC18 and named according to the deleted introns, e.g., Δ123, Δ12, Δ1, etc. In Δ1a (see Fig. 4), a 627-bp internal segment of intron 1 was removed by digestion of rpL32 with *Sac*II and *Kpn*I; in Δ1b, an additional 16 bp of 5′ sequence was removed by partial digestion of Δ1a with *Sph*I. Incompatible ends were blunted with the Klenow enzyme and religated. Constructs Δ123 · i and Δ123 · ir (see Fig. 3B) were made by first inserting a *Sma*I linker into Δ123 at the *Pvu*II site (position +3131 within exon IV) and then inserting into this *Sma*I site a 221-bp *Xmn*I fragment which spans the third and fourth constant-region exons of the mouse immunoglobulin μ gene (16). This fragment, kindly supplied by Martha Peterson, was inserted in both orientations by blunt-end ligation. For the series of constructs carrying insert A (see Fig. 5A), a 164-bp *Hae*II fragment extending from

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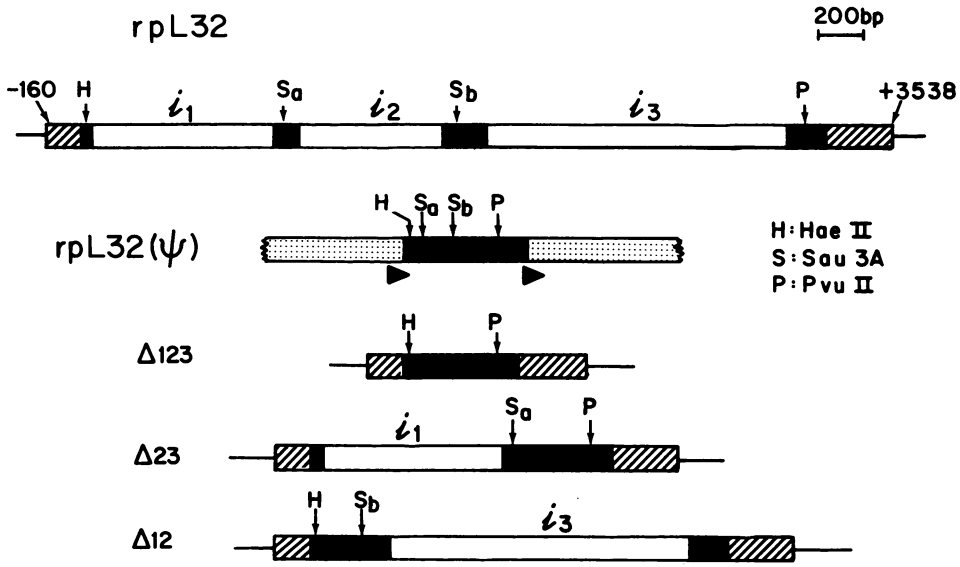

FIG. 1. Schematic representation of the expressed rpL32 gene, the unmutated processed pseudogene rpL32(ψ), and some representative intron-lacking constructs. Symbols: ■, exons; □, introns; ▨, ▩, flanking sequences; ▶, direct repeats that flank rpL32(ψ); ——, vector sequences. S_a and S_b, *Sau*3A sites in exons II and III, respectively; H, *Hae*II; P, *Pvu*II.

positions +12 to +175 was excised from rpL32 and inserted into Δ1, Δ12, Δ13, or Δ123 at selected sites. In Δ1 · A(+11), Δ12 · A(+11), Δ13 · A(+11), Δ123 · A(+11), and Δ123 · i · A(+11), it was inserted into the +11 *Hae*II site in its original orientation, thus reconstituting an intact exon I and the adjoining 129 bp of intron 1. In Δ1 · Ar(+11), it was inserted into the same *Hae*II site in the opposite orientation. In Δ1 · A(−160) and Δ1 · Ar(−160), it was blunt ended and inserted in both orientations into the vector junction of Δ1 at position −160. In Δ12 · A(+2685), it was inserted in its original orientation into the *Sma*I site in intron 3 of Δ12. An insertion at this site, which is located 928 and 375 bp, respectively, from the 5′ and 3′ splice junctions, presumably should not affect the splicing of intron 3 sequences. The correctness of the various constructions was verified by restriction analysis. The plasmids were grown in *Escherichia coli* HB101 and purified by banding twice in cesium chloride. The preparations used for transient transfection experiments consisted almost entirely of supercoiled DNA, as judged by agarose-gel electrophoresis.

DNA transfection and RNA isolation. Cultured COS-7 cells were transfected by the DEAE-dextran-chloroquine procedure and harvested from the plates 40 h later by trypsinization. Total-cell RNA and poly(A)$^+$ RNA were isolated as previously described (11). For isolation of cytoplasmic RNA, cell pellets were suspended in a solution containing 0.15 M NaCl, 2 mM $MgCl_2$, 20 mM Tris chloride (pH 7.4), and 0.5% Nonidet P-40 and gently vortexed; nuclei were removed by centrifugation for 5 min at 2,500 rpm. Supernatants were adjusted to 5% sodium dodecyl sulfate–5 mM EDTA–200 μg of heparin per ml and extracted twice with phenol-chloroform; the RNA was then precipitated with ethanol.

Analysis of RNA. Northern (RNA) blots of poly(A)$^+$ RNA were probed with nick-translated fragments containing the rpL32 exon sequences (Δ123) or a human β-actin cDNA sequence (11). S1 nuclease protection assays of cytoplasmic or total-cell RNA were done with a 5′-terminal rpL32 probe, an internal rpL32 probe, and a 5′-terminal rpS16 probe. The 5′-terminal rpL32 probe is a 243-bp *Sac*I-*Sau*3A fragment derived from Δ123 that contains 160 bp of 5′-flanking sequence, exon I, and a portion of exon II (Fig. 2A). An rpL32 transcript that is initiated at the normal cap site and properly spliced protects an 83-nucleotide segment of this probe. The internal rpL32 probe is a 351-bp *Pst*I-*Xho*II fragment that spans the intron 2-exon III splice junction. A properly spliced rpL32 transcript protects a 63-nucleotide segment of this probe (Fig. 2C). The rpS16 5′-terminal probe, a 244-bp *Ava*II fragment (24), yields a 68-nucleotide fragment when protected by properly initiated rpS16 transcripts.

Nuclear run-on experiments. Approximately 1.5×10^7 COS cells were cotransfected by electroporation (2) with linearized forms of an rpS16-*neo* gene and either L32, Δ1, Δ1 · A(+11), or pUC18. The rpS16-*neo* recombinant was constructed by fusing the promoter region of rpS16 (−179 to +29 [24; N. Hariharan and R. P. Perry, unpublished data]) to a promoterless *Hin*dIII-*Acc*I fragment derived from pSV2 *neo* (23). After selection in G418, about 20 transformant foci from each transfection experiment were pooled and propagated for 1 month in the presence of G418. Nuclei from 10^7 transformant cells were used for nuclear run-on experiments as described previously (17, 19). For each experiment, about 2×10^7 dpm of [^{32}P]RNA was hybridized with a Nytran strip containing dots or slots of the following samples of denatured DNA: a 1-kb *Sph*I-*Xmn*I fragment containing rpL32 intron 3 sequences (0.5 μg); a 2-kb *Bam*HI fragment derived from the human β-actin cDNA clone pHFβA-1 (14) (0.5 μg); and lambda bacteriophage (2 μg). After hybridization, washing (15 mM NaCl, 1.5 mM sodium citrate at 68°C), and RNase treatment, the Nytran strips were autoradiographed or directly scanned with an AMBIS radioanalytic system.

RESULTS

Intron 1 is essential for efficient rpL32 expression. Intron-deficient rpL32 genes were constructed by replacing selected segments of the normal gene with their appropriate counterparts from the unmutated processed pseudogene rpL32(ψ) (Fig. 1). One such construct, Δ123, contained the complete

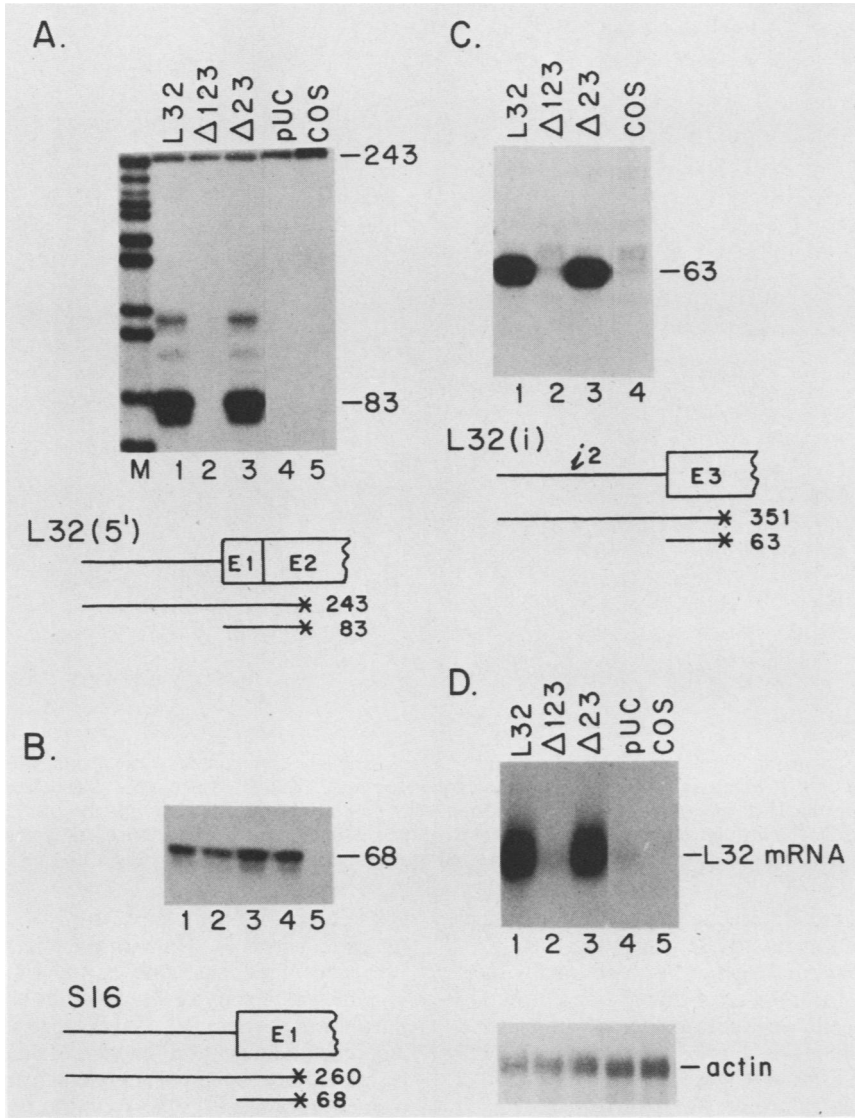

FIG. 2. Influence of intron 1 on expression of rpL32; assays of RNA from untransfected COS cells and from cells transfected with the indicated constructs. (A) S1 nuclease protection analysis of total-cell RNA with a 5'-terminal probe, rpL32(5') (diagrammed below). Lane M contains size markers made from *Msp*I-digested pBR322. (B) Expression of the cotransfected rpS16 gene assayed by S1 nuclease protection of a 5'-terminal rpS16 probe (diagrammed below). Lanes 1 through 5 are from the same experiment as the corresponding lanes in panel A. (C) Cytoplasmic RNA from an independent transfection experiment assayed by S1 nuclease protection analysis with an internal probe, rpL32(i) (diagrammed below). (D) Northern blot analysis of poly(A)$^+$ RNA from the experiment of panel A. Blot was hybridized with the rpL32 exon probe and then with a human β-actin probe to monitor the relative amount of RNA in each lane. E1, E2, and E3, Exons I, II, and III, respectively; *i*2, intron 2. Sizes (in nucleotides) of S1-protected fragments are shown to the right of panels A, B, and C. The faint bands migrating behind the major S1-protected fragments in panel A are due to an artifactual association of the probe with rpL32 transcripts. These bands were eliminated in later experiments by increasing the temperature of S1 nuclease digestion from 24 to 30°C.

set of exon sequences, 160 bp of 5'-flanking sequence, and 300 bp of 3'-flanking sequence but lacked all three introns. Another construct, Δ23, contained intron 1 in addition to the other sequences but lacked introns 2 and 3. These constructs were transfected into monkey kidney (COS-7) cells in parallel with the normal rpL32 gene (positive control) or the pUC12 vector (negative control), and transient expression was measured by S1 nuclease protection assay with either a 5' fragment spanning the cap site (Fig. 2A) or an internal fragment spanning intron 2 and exon III (Fig. 2C). Intact rpS16 genes were cotransfected with each construct and also assayed by S1 nuclease protection (Fig. 2B) to verify that transfection efficiencies and RNA yields were roughly equivalent. Expression was also monitored by a blot analysis of poly(A)$^+$ RNA with an exonic probe to identify the mature rpL32 mRNA and with a human actin probe to verify RNA equivalence (Fig. 2D).

Somewhat to our surprise, no expression of Δ123 could be detected (Fig. 2A, C, and D, lanes 2). The very weak signals observed with this construct were comparable to those of the mock (pUC)-transfected controls and untransfected COS cells (lanes 4 and 5), which we estimate to be less than 1% of the signal from the intact rpL32 gene (lane 1). In contrast, expression of the Δ23 construct (lane 3) was essentially the same as that of the normal rpL32 gene. These results indicate that intron 1, together with the exonic and appro-

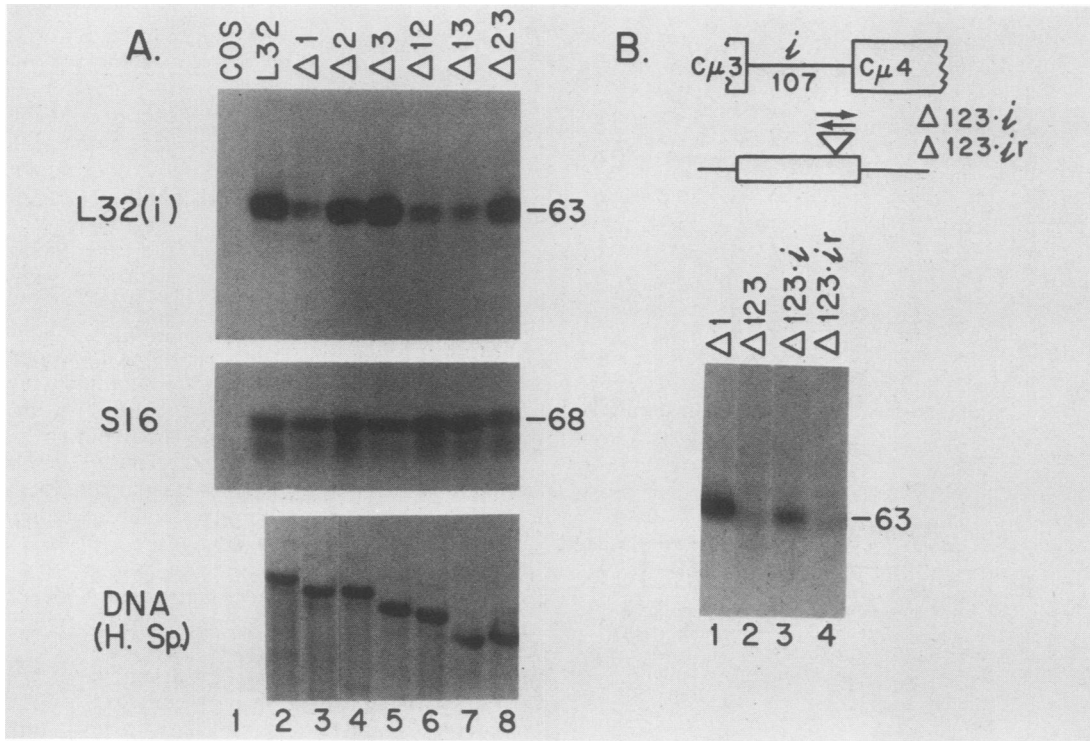

FIG. 3. Importance of intron splicing for rpL32 expression. (A) Cytoplasmic RNA from cells cotransfected with rpS16 and the indicated rpL32 constructs. rpL32 and rpS16 expression was analyzed as described for Fig. 2C and B, respectively. The bottom panel shows a Southern blot of plasmid DNA from the Hirt supernatant fractions digested with *Bam*HI and hybridized with the rpL32 exonic probe. (B) Cells transfected with constructs in which an intron-containing fragment from the Cμ3-Cμ4 region of the mouse heavy-chain locus was inserted into Δ123 in both orientations (diagrammed at the top). rpL32 expression was assayed as described for Fig. 2C.

priate flanking sequences, is both necessary and sufficient for maximum expression of the rpL32 gene.

The fact that intronless rpL32 genes are functionally inert was also indicated by the results of stable-transformation experiments. Mouse L cells were cotransfected with plasmid pSV2*neo* and either the normal rpL32 gene or the Δ123 construct. Stable transformants were isolated after G418 selection, and the RNA from more than 10 clones of each type was analyzed by RNA blotting. More than 80% of the rpL32 transformants contained at least five times more rpL32 mRNA compared with untransformed cells or cells transformed with plasmid pSV2*neo* only. In marked contrast, none of the Δ123 transformants showed any significant elevation of rpL32 mRNA over the endogenous level. Thus, both stably integrated and episomal rpL32 genes absolutely require introns for expression.

Introns have two distinct roles in rpL32 expression. The striking results of the foregoing experiments raised the question of why intron 1 plays such a critical role in the expression of rpL32. In an attempt to answer this question, we carried out a second set of transfection experiments with constructs lacking each of the individual introns or various pairs of introns (Fig. 3A). The relative activities of these constructs were quantitatively evaluated by using the expression of cotransfected rpS16 genes and assays of the content of transfected rpL32 plasmid DNA as appropriate reference controls. As might be anticipated from the result with Δ23 (see above), constructs lacking intron 2 or 3 alone (Δ2 or Δ3) were expressed at the maximum level (compare lanes 2, 4, 5, and 8 in Fig. 3A). However, constructs lacking intron 1, either alone (Δ1) or together with one of the other introns (Δ12 or Δ13), were expressed at a level of only 10 to 20% compared with expression of the intact rpL32 gene (lanes 3, 6, and 7). Thus, in the absence of intron 1 and the presence of at least one of the other introns, there is a residual basal activity which is not observed when all of the introns are absent. This result suggests that intron 1 has both a unique and a general function. The unique function, which is related to the presence of a transcriptional regulatory element (see below), was found to be responsible for a 5- to 10-fold increase in activity (Table 1). The general function, which can be provided by any of the introns, may be related to an intron-splicing requirement for maximum yield of rpL32 transcripts.

If there is indeed such a splicing requirement, it would appear to be rather nonspecific, since intron 2 or 3 can equivalently perform this putative function. To investigate the question of specificity further, we inserted a foreign intron into the exon IV region of the intronless construct Δ123 and compared the activity of this construct (Δ123 · i) with the activities of Δ1 and Δ123. A 107-bp intron derived from the mouse immunoglobulin μ heavy-chain gene could effectively substitute for the rpL32 introns, whereas this same sequence in reverse orientation (Δ123 · ir) had essentially no effect (Fig. 3B). This result strongly supports the idea that rpL32 expression is somehow dependent on the presence of a spliceable intron in its transcripts. Moreover, the nonspecificity of this phenomenon suggests some general requirement, such as the engagement of spliceosomes, for proper transcript utilization.

Localization of the functionally important intron 1 sequences. To identify which portion of intron 1 is responsible for its unique contribution to rpL32 expression, we examined the activities of two constructs containing deletions that

TABLE 1. Contribution of intron sequences to rpL32 expression[a]

Relative expression (% of maximum)	Gene construct(s)	Inferred deficiency
90-100	L32, Δ2, Δ3, Δ23, Δ1a, Δ1 · A(+11), Δ12 · A(+11), Δ13 · A(+11), Δ123 · i · A(+11)	None
20-40	Δ1b	Incomplete *tre*
	Δ1 · Ar(+11)	Disoriented *tre*[b]
10-20	Δ1, Δ12, Δ13, Δ123 · i	Lacking *tre*
	Δ1 · A(−160), Δ1 · Ar(−160), Δ1 · A(+2683)	Dislocated *tre*
<1	Δ123 · A(+11)	Lack of intron splicing
	Δ123, Δ123 · ir	Lack of intron splicing and *tre*

[a] Autoradiograms such as those illustrated in Fig. 2 through 5 were scanned with a densitometer, and the intensities were normalized to the value for the intact rpL32 gene. Exposure times were selected so that the densities were in the linear dose-response range. Expression of constructs bearing the foreign intron was slightly lower than that of counterparts bearing a ribosomal protein intron (e.g., Δ123 · i · A(+11) < Δ12 · A(+11) and Δ123 · i < Δ12). *tre*, Transcriptional regulatory element.

[b] Interpretation of this construct is complicated by the concomitant dislocation of exon I sequences.

removed approximately 80% of the intron 1 sequence (Fig. 4). The activity of a construct containing 27 bp of the 5′ region and 130 bp of the 3′ region of intron 1 (Δ1a) was nearly the same as that of the construct containing the entire intron 1 sequence (Δ23). In contrast, the activity of a construct containing the same 3′ region but only 11 bp of the 5′ region (Δ1b) was almost as low as that of the intron 1-lacking construct Δ1. The relative expression of this set of constructs was the same when assayed with either the L32(i) S1 probe (as in Fig. 4) or the L32(5′) probe (data not shown). In all cases, the L32(5′) probe yielded an 83-nucleotide protected fragment, which indicated that intron 1 was properly

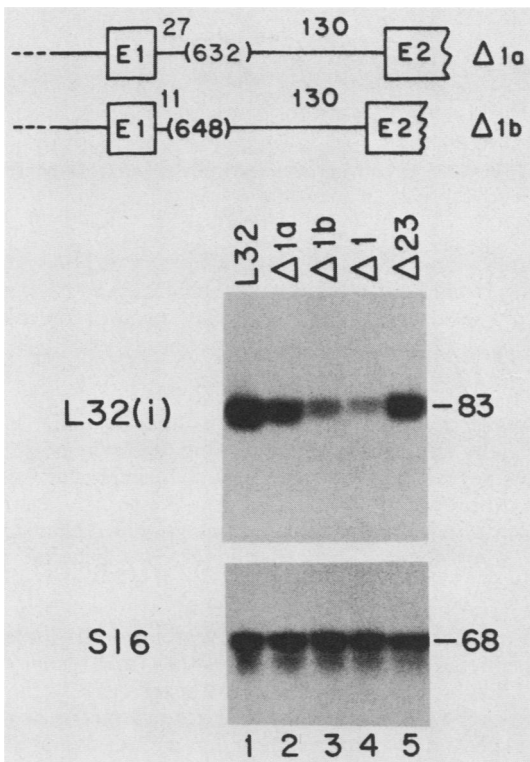

FIG. 4. Effect of a partial deletion of intron 1 sequence on rpL32 expression. Cells cotransfected with rpS16 and Δ1a or Δ1b (diagrammed at the top) or other selected rpL32 constructs were analyzed for rpL32 and rpS16 expression as described for Fig. 2C and B, respectively.

excised from the transcripts (Fig. 2A). Since intron 1 was efficiently removed from both Δ1a and Δ1b transcripts, there is no reason to suppose that these transcripts would have different stabilities. We conclude, therefore, that a functionally important regulatory element is located within the first 27 bp of intron 1 sequence.

The intron 1 regulatory element does not have the properties of a typical enhancer. To examine the properties of the intron 1 regulatory element, we excised a fragment, termed A, which consisted of 35 bp of exon 1 sequence and the first 129 bp of intron 1, and inserted it into various locations of Δ1 or Δ12 (Fig. 5A). When fragment A was inserted into its normal location [Δ1 · A(+11)], the level of expression was essentially equivalent to that of the normal rpL32 gene (Fig. 5B). This was not the case when fragment A was inserted in opposite orientation at the correct position [Δ1 · Ar(+11)] or 160 bp upstream of the cap site in either orientation [Δ1 · A(−160) and Δ1 · Ar(−160)] or in intron 3 at a position 375 bp upstream of the 3′ splice junction [Δ12 · A(+2685)]. Clearly, the intron 1 regulatory element does not have the position-independent properties that one would expect of a typical enhancer element. The extent of orientation dependence is difficult to judge from this experiment because the +12 to +46 exon I sequence is also dislocated in Δ1 · Ar(+11), and this dislocation could also contribute to the lowering of its activity.

In Δ1 · A(+11), the inserted intron 1 sequence lacks both the lariat branch site and a proper 3′ splice junction and is therefore unlikely to be excised from the RNA transcripts. Indeed, when these transcripts were analyzed by RNA blot analysis, a 0.78-kb component was observed (Fig. 5C). This component, which is about 0.13 kb larger than the normal mature L32 mRNA, presumably contains this unspliceable intron 1 sequence. In Δ1 · A(+11), the general splicing requirement could be fulfilled by intron 2 or 3. The presence of either of these introns together with fragment A was sufficient to give maximum levels of expression (Fig. 5D, lanes 5 and 6). As one might expect, when fragment A was inserted into Δ123, no expression was detected (Fig. 5D, lane 4) because of the lack of any spliceable intron sequence. However, when both fragment A and a foreign intron were inserted into Δ123 [Δ123 · i · A(+11)], expression was restored to about two-thirds the maximum level (Fig. 5D, lane 8). The finding that fragment A can increase expression by severalfold even though it does not satisfy the general RNA-splicing requirement implies that the intron 1 regulatory element acts at the transcriptional level.

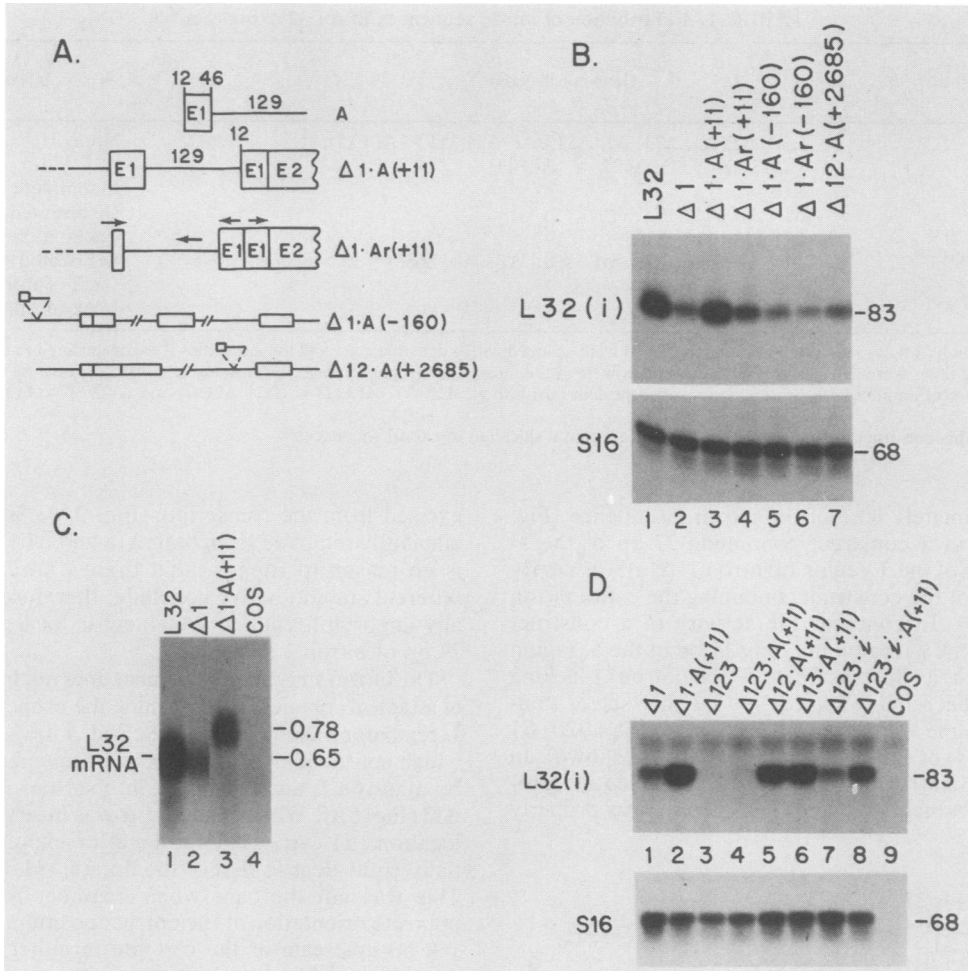

FIG. 5. Effect of orientation and translocation on the activity of the intron 1 regulatory element. (A) Diagram of the 164-bp *Hae*II fragment A and its insertion into various sites of the Δ1 and Δ12 constructs; (B) expression of these constructs and the cotransfected rpS16 controls, assayed as described in the legend to Fig. 2C and B; (C) Northern blot analysis of poly(A)$^+$ RNA from selected transfectants; (D) comparison of constructs with and without spliceable introns, assayed as described in the legend to Fig. 2B and C.

The intron 1 regulatory element functions at the transcriptional level. The transcriptional role of the intron 1 element was directly demonstrated by nuclear run-on analysis. For these experiments, COS cells were cotransfected with a selectable gene (*neo*) and either the wild-type or mutant rpL32 gene or a pUC vector. Nuclei isolated from a pool of about 20 independent G418-resistant transformants were used for the run-on analyses. The transcriptional activity of the transfected rpL32 genes was assayed with a 1-kb rpL32 intron 3 sequence. To monitor the overall RNA polymerase II activity in the different batches of nuclei, we assayed the transcriptional activity of the endogenous β-actin genes of the COS cells. Lambda phage DNA was used to monitor nonspecific hybridization.

Transcription was markedly reduced in the Δ1 mutant (Fig. 6A and B) and was close to the wild-type level in the Δ1 · A(+11) mutant (Fig. 6B). Southern blot analysis demonstrated that the various transformant pools contained similar numbers of rpL32 genes (Fig. 6C). Thus, we can conclude that the intron 1 element does indeed function at the transcriptional level. The sensitivity of these measurements is such that a residual activity of 10 to 20%, which was indicated by the S1 nuclease protection and Northern blot analyses, would not be distinguishable from the background observed with mock-transfected (pUC vector) controls.

DISCUSSION

A detailed analysis of the relative activities of the normal rpL32 gene and 20 variants lacking various intronic sequences revealed that introns play two important roles in rpL32 expression (Table 1). One role is uniquely ascribable to intron 1, which harbors a transcriptional regulatory element at its 5′ end. The other involves a general intron-splicing requirement that can be satisfied by any of the three rpL32 introns or even a foreign intron.

Additional evidence indicating that intron 1 contains a transcriptional regulatory element comes from recent experiments in which various 5′ portions of the rpL32 gene were linked to the chloramphenicol acetyltransferase gene (1). In these experiments, expression of a construct containing the first 26 bp of intron 1 sequence was significantly greater than that of a construct containing only the first 8 bp of intron 1 sequence. Consistent with its role in transcriptional regulation, this functionally important region of intron 1 was also shown to bind a nuclear factor (1). The binding site for

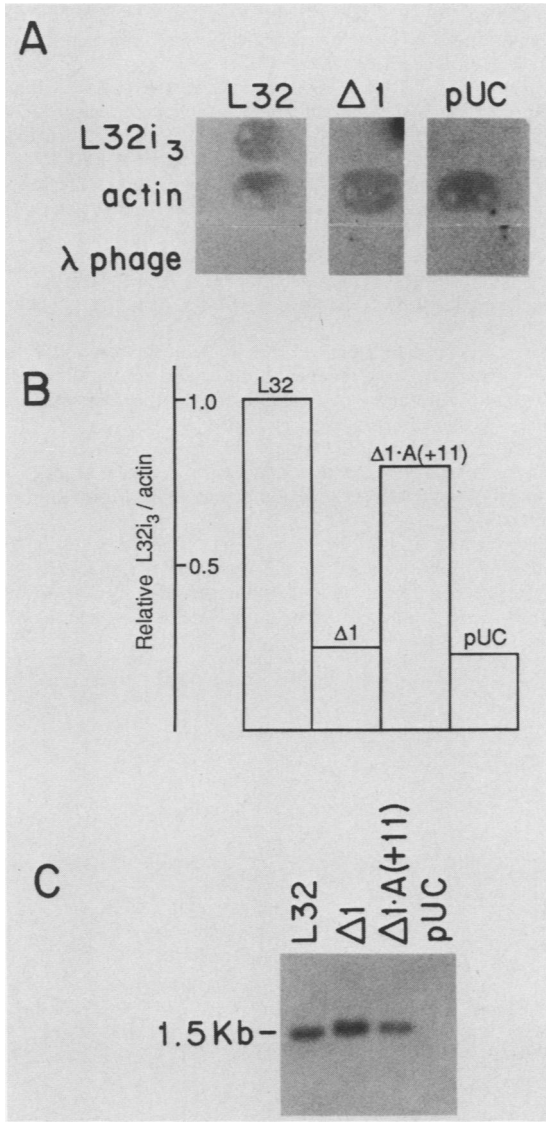

FIG. 6. Effect of intron 1 sequences on rpL32 transcriptional activity. (A) Autoradiogram of a nuclear run-on analysis of stably transformed COS cells containing the wild-type rpL32 gene (L32), the Δ1 mutant, or a pUC vector (mock transfection). The source of immobilized DNA is shown at the left. L32i_3, actin, and λ measure the signals from the transfected L32 genes, endogenous β-actin genes, and nonspecific background hybridization, respectively. (B) Results of a run-on experiment similar to that shown in panel A. In this experiment, radioactivity was measured directly by a 10-h scan with an AMBIS radioanalytic system. The measured L32i_3 and actin signals, 8 to 19 and 14 to 19 cpm, respectively, were corrected for background by subtracting the corresponding λ signals (4 to 7 cpm). The ratio of the L32i_3/actin net signals, normalized to that of the wild-type transformant, is shown as a bar graph. (C) Southern blot analysis of the rpL32 genes in the various transformants. DNA from each transformant pool was digested with *Eco*RI and probed with a 560-bp *Bst*E-*Sma*I fragment from intron 3 of rpL32. The 1.5-kb *Eco*RI fragment encompasses exon III and portions of introns 2 and 3 (10).

this factor might embrace the inverted repeat sequence CGGCGGCCATCCGCCG, which is located 13 to 28 bp downstream of the exon I-intron 1 junction (10). Our inability to demonstrate any activity of this element when it was translocated to other regions of the rpL32 gene suggests that the element probably does not function as a typical enhancer. A more likely possibility is that it is part of a complex promoter that encompasses approximately 200 bp of sequence spanning the cap site (1; Moura-Neto et al., in press). Within the context of this model, our results suggest that the promoter can function at about 10 to 20% of maximum efficiency without the contribution of the intron 1 element.

The fact that rpL32 expression has a general requirement for intron splicing is not unprecedented. Previous studies of several other genes have described negative effects of intron depletion, and, in at least some cases, these effects do not seem to be attributable to the loss of transcriptional elements (6, 7, 13, 15). On the other hand, not all genes contain introns, and intronless derivatives of some intron-containing genes seem to be expressed efficiently (8, 12, 20). Thus, this requirement can obviously be relaxed for some genes but not for others. For rpL32, an unspliceable intron sequence, such as occurs in the Δ123 · A(+11) construct, does not satisfy this requirement, which suggests that spliceosome assembly, or even the splicing event itself, may be necessary to engender the effect. Although the mechanistic details of this phenomenon are still obscure, it would seem prudent to acknowledge its possible importance when examining the expression of novel minigene constructs.

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