MOLECULAR AND CELLULAR BIOLOGY, May 1989, p. 2067–2074
0270-7306/89/052067-08$02.00/0

Vol. 9, No. 5

Localization of Transcriptional Regulatory Elements and Nuclear Factor Binding Sites in Mouse Ribosomal Protein Gene rpL32

MICHAEL L. ATCHISON, ODED MEYUHAS,† AND ROBERT P. PERRY*

Institute for Cancer Research, Fox Chase Cancer Center, 7701 Burholme Avenue, Philadelphia, Pennsylvania 19111

Received 19 November 1988/Accepted 27 January 1989

The DNA sequences required for expression of the ribosomal protein gene rpL32 were identified by transient-expression assays of chimeric rpL32-chloramphenicol acetyltransferase genes. These studies showed that maximal rpL32 expression requires sequences in a 150- to 200-base-pair region spanning the transcriptional start site. Three discrete regions of importance were identified: one between positions −79 and −69 and two others located downstream of the transcriptional start site. Progressive 5′ or 3′ deletions caused stepwise decreases in expression, which suggested a complex interplay of redundant or compensatory elements. Gel mobility shift assays were used to identify *trans*-acting nuclear factors which bind to segments of the rpL32 promoter that are known to be important for transcription. Evidence for several distinct nuclear factors is presented. The binding sites for these factors were localized to the following regions: −79 to −69, −36 to −19, −19 to +11, +11 to +46 in exon I, and within the first 31 base pairs of intron 1. One of these factors may bind to multiple sites within the promoter region. Interestingly, the factor that binds to a sequence motif in the first exon also binds to similar motifs in a comparable region of the c-*myc* gene.

The ribosomal proteins are structurally diverse and evolutionarily unrelated to each other, yet the genes that encode them are expressed with very similar efficiencies, as indicated by the relatively uniform abundance of various ribosomal protein mRNAs and by the similar RNA polymerase loading of different ribosomal protein genes (11, 12; D. E. Kelley and R. P. Perry, unpublished observations). What is the basis for this similarity? Presumably, the transcriptional efficiency of a ribosomal protein gene should ultimately be determined by the characteristics of its particular set of regulatory elements and by the cellular content of *trans*-acting factors that interact with these elements. To improve our understanding of these elements and factors, we have sought to define the DNA sequences that constitute the complete promoter of the ribosomal protein gene rpL32 and to identify the nuclear factors that bind to the functionally important sequences. Using a series of constructs in which selected portions of the rpL32 promoter region were linked to a chloramphenicol acetyltransferase (CAT) reporter gene, we observed that maximal expression requires sequences in a 150- to 200-base-pair (bp) region spanning the transcriptional start site, including sequences within exon I and intron 1. Moreover, we show that there are several discrete nuclear factor binding sites within this region, at least one of which recognizes a factor that is also bound by the *myc* gene promoter. These findings, together with previous and other parallel studies of rpL32 expression (2, 5; R. Moura-Neto, K. P. Dudov, and R. P. Perry, Proc. Natl. Acad. Sci. USA, in press), have delineated the complex set of sequences that determine the efficiency of rpL32 transcription. The unusual organization of this promoter may enable it to function with a similar efficiency in a wide variety of cell types.

* Corresponding author.
† Present address: Department of Developmental Biochemistry, Hadassah Medical School, The Hebrew University, Jerusalem, Israel 91010.

MATERIALS AND METHODS

Plasmid constructions. Plasmid p106 is composed of an *Eco*RI-*Sma*I DNA segment derived from pSVOCAT containing the CAT gene linked to the simian virus 40 (SV40) splice and polyadenylation signals (7), inserted into the *Eco*RI-*Sma*I sites of pUC13. Various rpL32 DNA segments (4) were inserted at the multiple cloning site just upstream of the CAT sequences. For some rpL32-CAT constructs, appropriate rpL32 DNA fragments were made blunt with Klenow polymerase and inserted into the unique *Sma*I site in the p106 polylinker region. These constructs, and the restriction endonuclease sites defining their boundaries, are as follows: −159,+195 (*Acc*I to *Ava*I); −159,+72 (*Acc*I to *Sac*II); −69,+72 (*Xmn*I to *Sac*II); and −159,+12 (*Acc*I to *Hae*II). For construct −79,+72, the 5′ boundary was produced by BAL 31 resection, followed by addition of a *Bam*HI linker (prepared by K. Dudov). To prepare the −159,+64 and −159,+54 constructs, a segment of the rpL32 gene spanning sequences −159 to +72 was excised by *Hin*dIII-*Sac*II digestion and purified by electrophoresis through a 6% polyacrylamide gel. For the −159,+64 clone, this purified DNA fragment was cut at the *Sal*I site in the polylinker region on the 5′ side of the rpL32 sequences and at the *Hae*III site at position +64 in the rpL32 gene. This *Sal*I-*Hae*III DNA segment was purified by electrophoresis through a polyacrylamide gel and cloned into the *Sal*I-*Sma*I sites of p106. For the −159,+54 construct, the −159 to +72 DNA fragment was partially cleaved with *Hin*fI, and the appropriate-size DNA segment was purified by gel electrophoresis and cloned into the *Xba*I site of p106, which had been made blunt by filling in. For construction of the −36,+72 construct, a pUC12 subclone containing sequences spanning −36 to +72 was linearized at the *Eco*RI site just 3′ of the rpL32 sequences, filled in with Klenow polymerase, and blunt-end ligated to a filled-in 1.6-kilobase *Bam*HI DNA fragment derived from p106 containing the CAT gene linked to the SV40 splice and polyadenylation sequences. Crucial

sequence boundaries were verified by DNA sequence analysis.

Cell culture and DNA transfection. S194 mouse plasmacytoma cells were obtained from the American Type Culture Collection (Rockville, Md.) and maintained in Dulbecco modified Eagle medium (GIBCO Laboratories, Grand Island, N.Y.) containing 10% horse serum. Cells were transfected by the DEAE-dextran (Pharmacia Fine Chemicals, Piscataway, N.J.) procedure according to the method of Grosschedl and Baltimore (8), followed by a 30-min treatment with chloroquine diphosphate (Sigma Chemical Co., St. Louis, Mo.). Cells were harvested between 40 and 46 h after transfection. Preparation of cell extracts, CAT assays, and thin-layer chromatography were performed as described by Gorman et al. (7).

Nuclear factor binding assays. Nuclear factor binding assays and polyacrylamide gel electrophoresis were performed essentially as described by Singh et al. (14). Each assay contained 8 μg of nuclear extract protein (prepared by the method of Dignam et al. [3]), 0.1 to 0.5 ng of the ^{32}P-labeled DNA fragment to be analyzed, and 3.2 μg of poly(dI-dC) · poly(dI-dC) (Pharmacia). Specific competitor DNA fragments, purified by electrophoresis on polyacrylamide gels followed by electroelution, were added to the binding reactions in the molar excesses indicated. Labeled rpL32 DNA fragments derived from the region spanning −36 to +77 were isolated from a rpL32 subclone beginning at position −36. This plasmid was digested with restriction endonuclease *Xba*I (which cuts in the polylinker region just 5′ of the rpL32 sequences), labeled by filling in with Klenow polymerase and [α-^{32}P]dCTP, and then cleaved with either *Sac*II, *Hae*III, *Hin*fI, or *Hae*II to produce DNA fragments beginning at position −36 and extending to position +77, +64, +49, or +11, respectively. Alternatively, a purified DNA fragment spanning sequences −36 to +77 was labeled at both ends by filling in with Klenow polymerase and [α-^{32}P]dCTP and then cleaved with *Hha*I or *Hae*II to produce labeled DNA fragments spanning −36 to −19, −36 to +11, or +11 to +77. Labeled DNA segments beginning at position −79 or −69 were prepared from construct −79,+72 or −69,+72, respectively. Each plasmid was cleaved at the *Bam*HI site 5′ of the rpL32 sequences, labeled by filling in with Klenow polymerase and [α-^{32}P]dCTP, and then digested with restriction endonuclease *Hin*fI. The resulting labeled DNA fragments spanning −79 to −41 or −69 to −41 were purified by electrophoresis on a polyacrylamide gel and isolated by electroelution. The immunoglobulin V_κ19 fragment was isolated from the 5′ flank of the MPC11 κ gene (9). The fragments from the *myc* gene promoter were kindly supplied by Kenneth Marcu.

RESULTS

Definition of the functionally important elements of the rpL32 promoter. In the experiments reported here, various portions of the rpL32 promoter region were linked to a promoterless CAT gene and transfected into mouse plasmacytoma cells, and their effectiveness was evaluated by measurements of CAT activity. These experiments were carried out concurrently with other studies in this laboratory (2; Moura-Neto et al., in press) in which rpL32 genes bearing deletions of 5′-flanking or internal sequences were transfected into monkey kidney cells and their transient expression was assayed by S1 nuclease protection and Northern (RNA) blot analyses. As will be seen, a comparison of the results from these two types of experiment is very helpful in determining the relative contributions of different segments of this complex promoter to its overall efficiency. This is particularly important when evaluating the role of internal sequences which, in principle, could contribute at both transcriptional and post transcriptional levels. Furthermore, the use of murine cells for these experiments ensures that the results of the other studies were not influenced by possible species-specific variations in the complement of transcription factors.

The rpL32 promoter segments were inserted into a pUC13-based vector (p106) containing the CAT gene linked to the SV40 splice and polyadenylation signals derived from pSVOCAT (Fig. 1A). These chimeric constructs were introduced into S194 plasmacytoma cells, and activity was measured in transient-expression assays. Initial experiments showed that rpL32 promoter sequences spanning the −159 to +72 region (relative to the cap site) were more active in driving CAT expression than was the Rous sarcoma virus long terminal repeat or the SV40 enhancer-promoter combination (data not shown). We therefore defined the activity of the −159 to +72 construct as 100%. Progressive 5′ deletions beginning at −159 and extending to −36 were tested. Deletion of 5′ sequences to −79 had only a slight effect on CAT activity, whereas deletion to −69 reduced activity to about 40% (Fig. 1B and C). Further deletion to −36 lowered activity to approximately 25%. These results are in close agreement with those obtained with the intact rpL32 gene (82, 42, and 28% for the −79, −69, and −36 mutants, respectively [Moura-Neto et al., in press]), thus confirming the reliability of the chimeric CAT constructs for assessing the relative contributions of the various rpL32 regulatory sequences.

To determine the 3′ boundary of the rpL32 promoter, the 5′ flank was maintained at position −159, and progressive 3′ deletions were prepared starting at position +195 and ending at +11. The AUG translational start codon in the rpL32 gene lies within exon II, and the exon I-intron 1 boundary is at position +46. In all constructs, the first AUG codon is the CAT initiator codon. Only a small decrease in activity was observed as sequences from +195 to +64 were deleted, which indicated that these sequences are of minor importance for the rpL32 promoter activity (Fig. 1B and C). In contrast, a deletion to position +54 caused a rather abrupt drop in activity, and further deletion to +12 decreased expression to less than 10% of the maximum value. The fact that activity declined sharply when the intron 1 sequences between +54 and +64 were removed suggests that the boundary of an important element lies within this region of intron 1. The large decrease in activity observed when sequences between +12 and +54 were deleted indicates that there is another important element in this region. These conclusions corroborate those drawn from independent experiments which examined the effects of segmental deletions of exon I (Moura-Neto et al., in press) and intron 1 sequences (2) on rpL32 expression. Implication of the intron 1 element in transcriptional regulation was directly demonstrated by nuclear run-on assays (2). Thus, a requirement for exon I and intron 1 sequences is evident irrespective of whether these sequences are linked to a CAT gene or to the remainder of the complete rpL32 gene. These findings, together with the factor binding data presented below, indicate that the internal sequences have a role in transcriptional regulation. The two internal elements and those in the 5′ flank presumably constitute the complex promoter of the rpL32 gene.

Evidence for *trans*-acting factors that bind to functionally

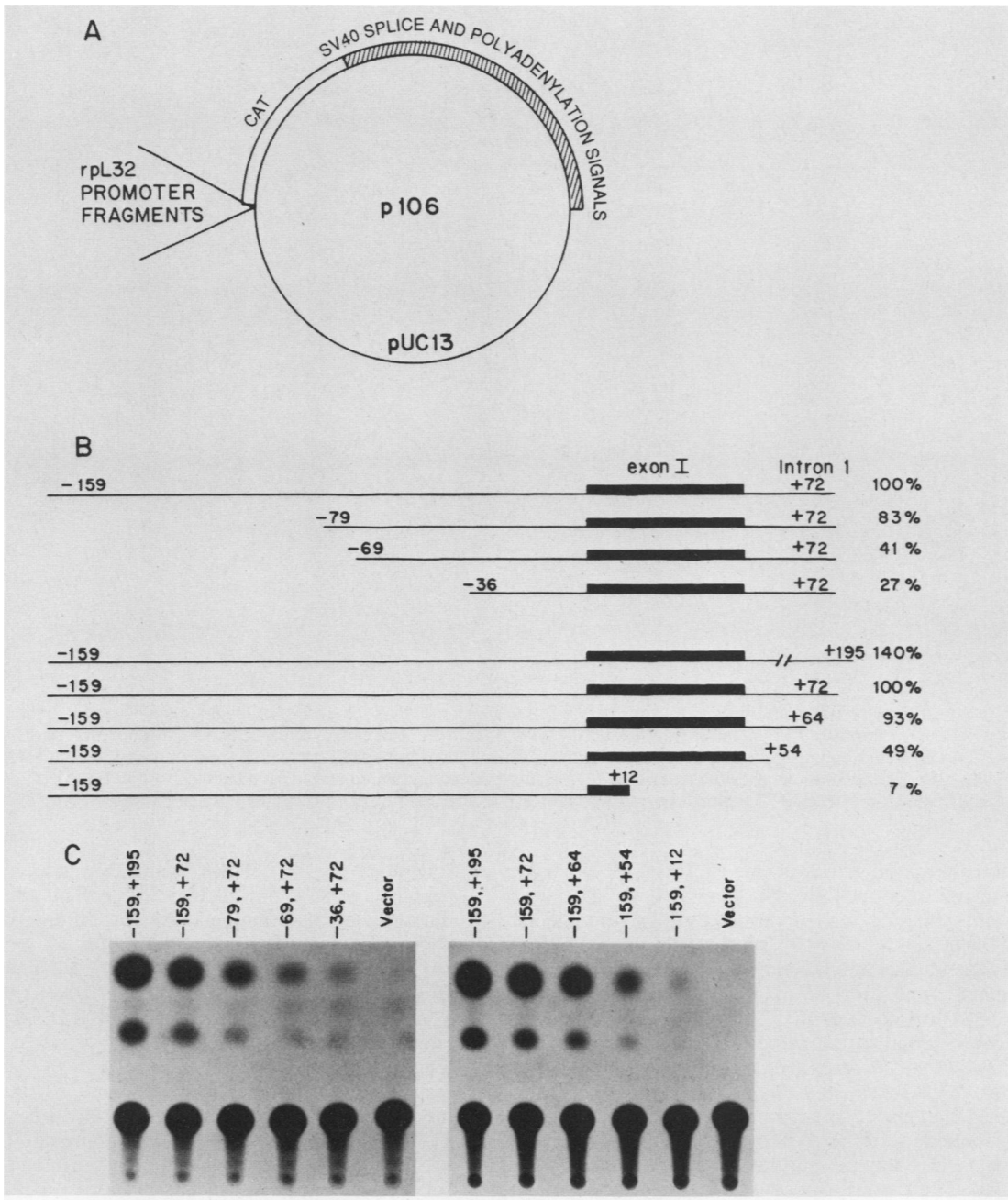

FIG. 1. Promoter activities of various rpL32 DNA segments. (A) Map of plasmid p106. Symbols: ▭, promoterless CAT gene; ▨, sequences containing the SV40 splice and polyadenylation signals; ——, pUC13 DNA sequences. The position where various rpL32 DNA fragments were inserted upstream of the CAT gene is indicated. (B) Diagram of rpL32 DNA segments used in each construct. Symbols: ——, 5′-flanking and intron 1 sequences; ▬, exon I. Endpoints of the rpL32 DNA sequences in each construct are indicated, and the average activity of each construct, normalized to the value for −159,+72, is shown at the right. Data for each construct were obtained from three to five separate transfection experiments. The activity of each construct was measured by directly counting the reaction products with an AMBIS Radioisotope Scanning System II after thin-layer chromatography. This value was then corrected for variations in transfection efficiencies by measurements of the amount of plasmid DNA in the Hirt supernatant fractions. (C) Typical CAT assay with each of the rpL32-CAT constructs. Acetylated derivatives of [^{14}C]chloramphenicol were fractionated by thin-layer chromatography and visualized by autoradiography. Above each lane is shown the rpL32-CAT construct used for transfection.

important rpL32 sequences. The identification of several distinct regions important for the control of rpL32 gene expression encouraged us to look for *trans*-acting factors that interact with these DNA sequences. To assess the ability of sequences between positions −79 and −69 to recognize *trans*-acting factors, DNA segments containing sequences spanning −79 to −41 or −69 to −41 were end labeled with ^{32}P and used in gel mobility shift assays (6). Incubation of each DNA fragment with a nuclear extract prepared from S194 plasmacytoma cells yielded striking results. The −79 to −41 DNA segment bound a nuclear factor resulting in the appearance of a slower-migrating band

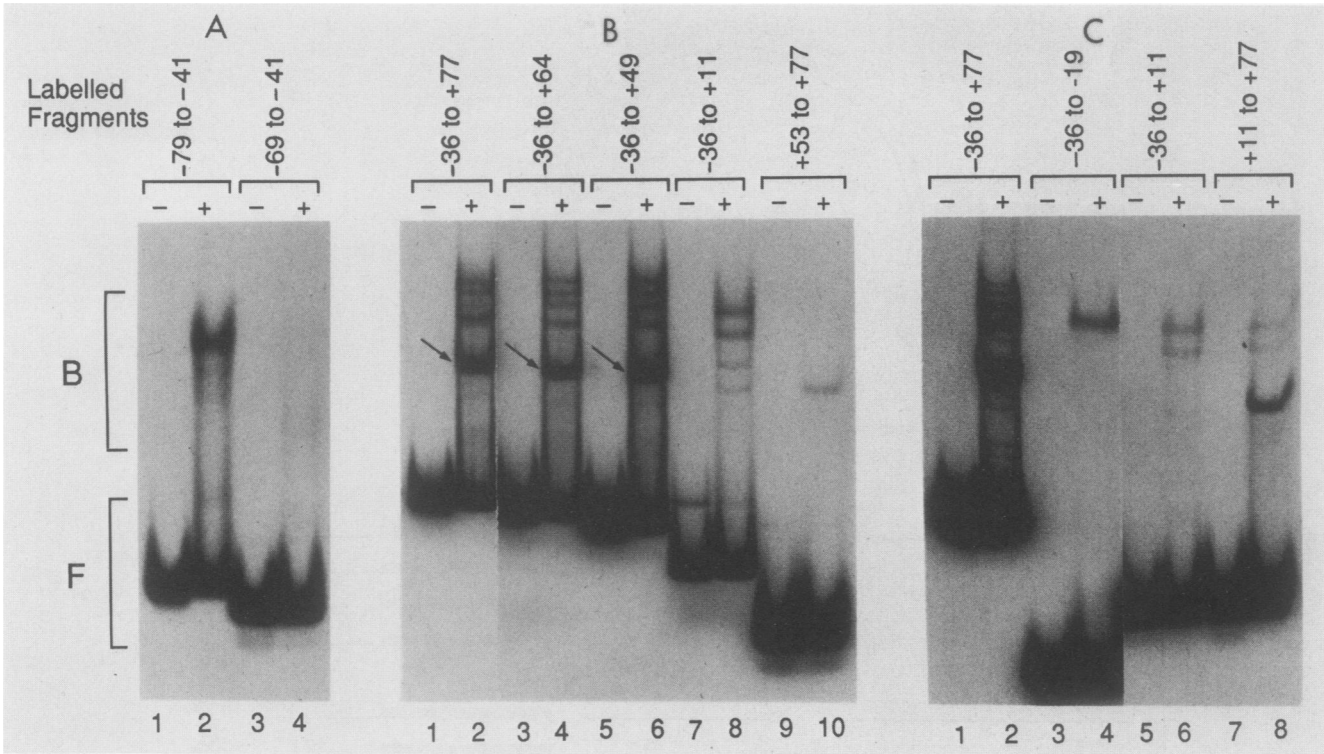

FIG. 2. Nuclear factor binding assays with various rpL32 DNA fragments. End-labeled DNA fragments derived from the rpL32 gene were incubated with (+) or without (−) nuclear extract proteins isolated from S194 plasmacytoma cells, electrophoresed on 4% polyacrylamide gels, dried, and visualized by autoradiography. The positions of free (F) and bound (B) bands are indicated on the left. Nucleotide positions corresponding to the 5′ and 3′ boundaries of each labeled DNA fragment are indicated above each lane. (A) Analysis of the −79 to −41 region; (B and C) analysis of the −36 to +77 region. Arrows in panel B indicate positions of the major intense bands.

upon electrophoresis, whereas the −69 to −41 DNA segment remained unbound (Fig. 2A, lanes 1 to 4). The specificity of this binding was demonstrated by the efficient competition of a −159 to +77 fragment that contained the −79 to −69 sequence and the virtual absence of competition by a fragment from the −36 to +77 region that lacked the −79 to −69 sequence (Fig. 3C). Thus, a *trans*-acting nuclear factor clearly bound to the same DNA sequence (AGCCG GAAGTG) that was determined to play a role in rpL32 gene expression. We have termed this factor α (Fig. 4).

A similar analysis was performed with the DNA segment spanning sequences in the −36 to +77 region. This segment yielded a very complex pattern of DNA-nuclear factor interactions. The pattern consisted of a major intense band (arrows in Fig. 2B) and four to five fainter bands of slower mobility (Fig. 2B, lanes 1 and 2). These interactions appeared to be specific because the factors binding to this region were outcompeted by an excess of homologous unlabeled fragment but not by a fragment from the promoter region of an immunoglobulin κ gene, $V_κ19$ (Fig. 3A). The quality of the $V_κ19$ competitor was verified by its effectiveness against the homologous labeled $V_κ19$ fragment (Fig. 3B). In an attempt to simplify the pattern, smaller DNA segments were used in the assay. The binding patterns of fragments spanning −36 to +64 and −36 to +49 (Fig. 2B, lanes 3-6) were similar to that of the −36 to +77 fragment. However, since the complexity of the pattern might obscure a single-band difference, we decided to examine the functionally important region of intron 1 more carefully with a DNA segment containing the +53 to +77 sequence. A single band of slower mobility was indeed observed with this fragment (Fig. 2B, lanes 9 and 10), which indicated the presence of a bound nuclear factor. The specificity of this binding was also demonstrated by differential competition with a fragment containing the +53 to +77 sequence and the unrelated $V_κ19$ fragment (Fig. 3F). We have termed this factor ε (Fig. 4).

When assays were performed with a DNA segment spanning sequences −36 to +11, the major intense band was no longer observed (Fig. 2B, lanes 7 and 8). The binding site responsible for the major intense band was thus localized to the +11 to +49 region, and the factor was designated δ (Fig. 4). The location of the δ-factor binding site correlates well with the expression data indicating that loss of sequences between +12 and +54 results in at least a 10-fold reduction in expression. The residual set of retarded bands observed with the −36 to +11 fragment also appeared to represent specific binding to rpL32 promoter sequences, since this binding was outcompeted by a fragment that contained the −36 to +11 sequence but not by the $V_κ19$ fragment (Fig. 3E). Further reduction of the DNA size to a fragment spanning −36 to −19 yielded a single band (Fig. 2C, lanes 3 and 4), which was outcompeted by homologous but not by nonhomologous sequences (Fig. 3D). This finding correlates well with previous data indicating that sequences between −36 and −13 are essential for rpL32 expression (5). The factor binding to the −36 to −19 region was termed β, and the one or more factors binding to the −19 to +11 region were collectively designated γ (Fig. 4).

Assays performed with a DNA segment spanning sequences +11 to +77 yielded a major intense band as well as two fainter, slower-migrating bands (Fig. 2C, lanes 7 and 8).

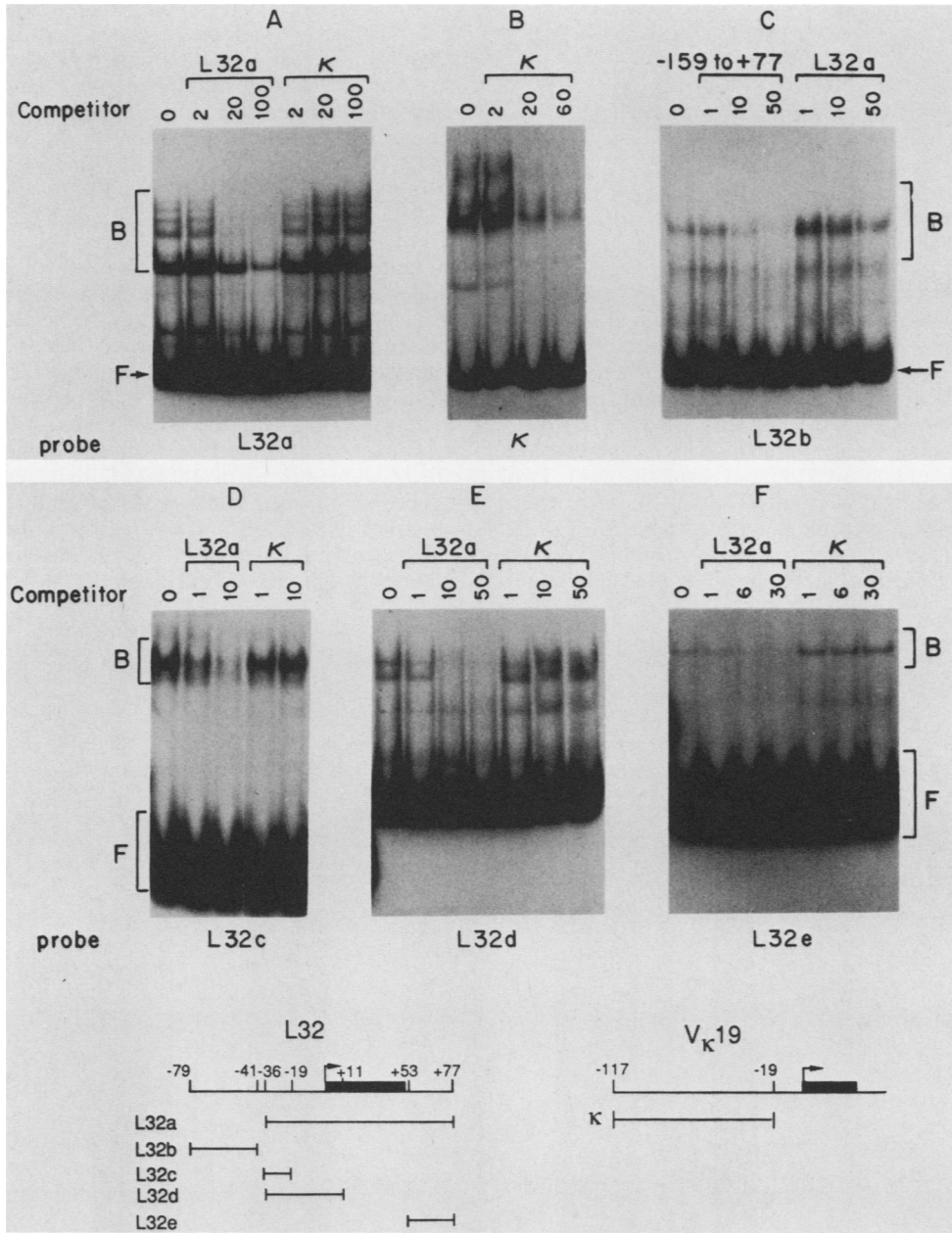

FIG. 3. Specificity of factor binding to segments of the rpL32 promoter. Various labeled fragments, indicated below each panel and diagrammed at the bottom, were used in factor binding assays together with the indicated molar excess of unlabeled competitor fragments shown above each panel.

The similarity in the pattern of slower-migrating components observed with the nonoverlapping DNA segments spanning −36 to +11 and +11 to +77 (compare lanes 6 and 8 of Fig. 2) suggested that these components might have resulted from binding of the same factor to more than one DNA site. To investigate this possibility, the labeled −36 to +77 DNA fragment was assayed in the presence of an unlabeled −36 to +11 fragment added as competitor. Although this competitor did not eliminate the band ascribed to the δ factor, it completely abolished the fainter, slowly migrating bands ascribed to the β and γ factors (Fig. 5, lanes 2 and 3). When the competition experiment was repeated with unlabeled competitor spanning sequences +11 to +77, the δ band and all but one of the β-γ bands was lost (Fig. 5, lanes 4 and 5). These results indicate that binding sites for a common factor or for factors with similar DNA-binding specificities lie both upstream and downstream of +11. In summary, there are binding sites for several nuclear factors in the region of the rpL32 promoter. The locations of the binding sites for these factors (Fig. 4) generally correlate well with the functionally important regions of the rpL32 promoter. Among these factors, α is clearly unique (Fig. 3B) and δ does not correspond to either β or γ (Fig. 5A). There may be some overlap between ε and β or δ, although further experiments will be required to define the nature of this overlap.

DNA sequences within the c-*myc* gene compete for rpL32 binding. The unusual organization of the rpL32 promoter resembles that of the c-*myc* promoter in that both genes

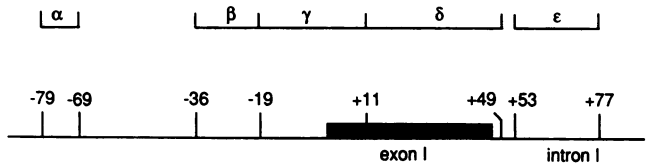

FIG. 4. Summary of binding assay data. Symbols: ▬, rpL32 first exon; ——, 5′-flanking and intron 1 sequences. Relevant nucleotide positions are indicated; nuclear factors that bind to specific regions of the rpL32 gene are designated α, β, γ, δ, and ε.

contain regulatory sequences within exon I and at the exon I-intron 1 boundary (18). To assess whether sequences within this region of the c-*myc* gene bind to the same factors that interact with the rpL32 promoter, competition experiments were performed with the labeled −36 to +77 rpL32 segment and unlabeled segments of the c-*myc* gene that are known to be involved in transcriptional regulation (18). Two segments, defined by a 179-bp *Hae*III-*Xho*I fragment from the 3′ region of the c-*myc* exon and a 51-bp *Xho*I-*Bgl*II fragment spanning the exon-intron boundary, competed very effectively for the δ-factor (Fig. 5, lanes 6 to 9). Methylation interference footprinting of the δ-factor binding site indicates that this factor interacts with the motif CTGCCATC at positions +30 to +37 in the rpL32 first exon (Moura-Neto et al., in press). Interestingly, the *myc* gene segments that efficiently compete for this binding factor contain related sequences (CTGCCCGC in the *Hae*III-*Xho*I fragment and CAGCCTTC in the *Xho*I-*Bgl*II fragment).

DISCUSSION

The results presented here have helped localize the DNA sequences that control rpL32 gene expression and have provided evidence for *trans*-acting nuclear factors that interact with these sequences. Since identification of the 3′ boundary of the rpL32 control sequences relied on transient-expression assays with rpL32-CAT fusion genes that produce transcripts with variable 5′ ends, it is not possible to rigorously exclude the possibility that some of the observed effects were due to posttranscriptional events. However, this possibility seems unlikely for several reasons. First, the construct most likely to be adversely effected by stability and processing differences due to inclusion of intron sequences within the mature mRNA is −159,+195. Tran-

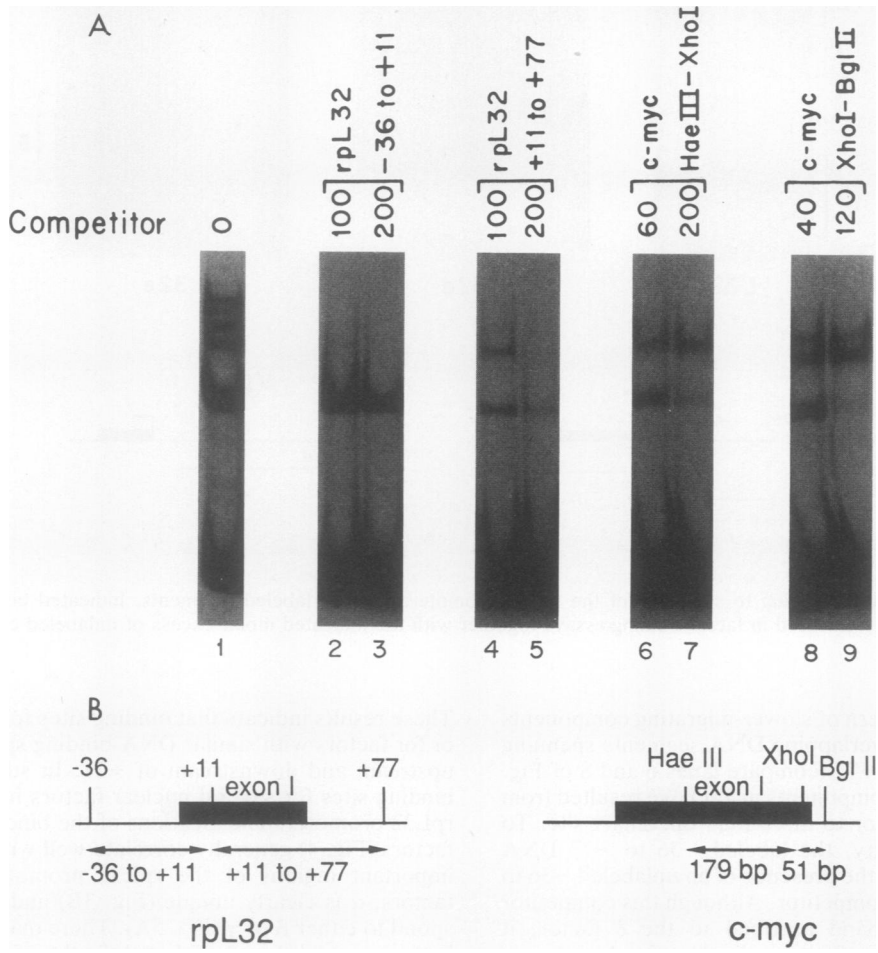

FIG. 5. Competition binding assays with rpL32 or c-*myc* DNA fragments. (A) A labeled rpL32 DNA fragment spanning residues −36 to +77 was incubated with nuclear extract proteins in the presence of unlabeled competitor DNA fragments derived from either the rpL32 gene or the c-*myc* gene. The competitor DNA fragments used and the molar excess of each competitor are listed above each lane. (B) Positions in the rpL32 and c-*myc* genes from which the competitor DNA fragments were derived. Symbols: ——, 5′-flanking and intron 1 sequences; ▬, exon I.

scripts produced by this construct contain the entire first exon, a 5′ splice site, 149 bp of intron sequence, and no 3′ splice site, yet this construct was the most active in our study. Removal of intron sequences to give transcripts more closely approximating normal rpL32 mRNA (−159,+54) results in lowered activity. Additional removal of all rpL32 intron sequences (−159,+12) yields the least active construct. Thus, inclusion of sequences most likely to exert adverse effects on RNA stability and processing actually results in higher expression. This inverse relationship strongly suggests that these sequences play a role in transcriptional regulation. Second, the ability of these internal DNA sequences to bind *trans*-acting nuclear factors is also consistent with a role in transcriptional control. Indeed, in a recent study of another mouse ribosomal protein gene, rpL30, site-specific mutagenesis of an exon I sequence resulted in a concomitant decrease in factor binding ability and promoter activity (N. Hariharan, D. E. Kelley, and R. P. Perry, manuscript submitted). Finally, the importance of intron 1 sequences for transcription was directly demonstrated by nuclear run-on experiments (2).

One novel aspect of the rpL32 control sequences is their internal location with respect to the rpL32 cap site. The boundary of these sequences lies about 80 bp beyond the site of transcriptional initiation. Although it is atypical for polymerase II promoters to contain functional information 3′ of the transcriptional start site, this organization is not without precedent. As mentioned above, the c-*myc* promoter also has regulatory sequences within the first exon and at the exon-intron boundary. For the c-*myc* gene, these sequences do not behave as an enhancer because they must be 3′ of the transcriptional start site and in the correct orientation (18). Similarly, internal rpL32 control sequences are nonfunctional when moved away from their normal locations (2). Thus, these control sequences appear to be an integral part of the rpL32 promoter. The similar organization of the rpL32 and c-*myc* promoters and the apparent ability of these promoters to interact with the same *trans*-acting nuclear factor suggest that these two genes possess some common regulatory features.

The very complex binding pattern of the rpL32 promoter region and the relatively gradual loss of activity during progressive 5′ or 3′ deletions suggest that this promoter is regulated by a complex assortment of factors. We have identified the regions within the rpL32 promoter where some of these factors bind. However, it is not possible at present to definitively assign the binding sites for all factors binding in the −80 to +80 region. The binding pattern may be complicated by differential occupancy of binding sites, by posttranslational modifications of some nuclear factors, or by the binding of distinct factors with similar binding specificities to the same DNA site. Indeed, the latter situation has been observed for several nuclear factor binding sites (1, 10, 13, 15–17). The apparent ability of different regions of the promoter to bind to the same nuclear factor, or to factors with similar binding specificities (e.g., the sequences 5′ and 3′ of +11), is reminiscent of the SV40 early promoter, which contains multiple Sp1 binding sites. The complex and slightly redundant organization of the rpL32 promoter may relate to its ability to be expressed in all cell types. Thus, cells that are deficient in some *trans*-acting factors might still be able to express the gene efficiently because of the presence of other compensatory factors.

ACKNOWLEDGMENTS

We thank Kalin Dudov for providing several cloned segments of the rpL32 gene, Mike Gilmen for plasmid p106, and Kenneth Marcu for the c-*myc* gene fragments.

This research was supported by grant DCB-84-136-9 from the National Science Foundation, Public Health Service grants AI-17330-07 and CA-06927 from the National Institutes of Health, and an appropriation from the Commonwealth of Pennsylvania. M.L.A. was a National Institutes of Health postdoctoral fellow (Public Health Service grant AI-0709-03). O.M. acknowledges support from the Stanley Reimann Fund of the Institute for Cancer Research and from the University of Pennsylvania-Israel Exchange Program.

LITERATURE CITED

1. **Baldwin, A. S., Jr., and P. A. Sharp.** 1988. Two transcription factors, NF-κB and H2TF1, interact with a single regulatory sequence in the class I major histocompatibility complex promoter. Proc. Natl. Acad. Sci. USA **85**:723–727.
2. **Chung, S., and R. P. Perry.** 1989. Importance of introns for expression of mouse ribosomal protein gene rpL32. Mol. Cell. Biol. **9**:2075–2082.
3. **Dignam, J. D., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11**:1475–1489.
4. **Dudov, K. P., and R. P. Perry.** 1984. The gene family encoding the mouse ribosomal protein gene L32 contains a uniquely expressed intron-containing gene and an unmutated processed gene. Cell **37**:457–468.
5. **Dudov, K. P., and R. P. Perry.** 1986. Properties of a mouse ribosomal protein promoter. Proc. Natl. Acad. Sci. USA **83**: 8545–8549.
6. **Fried, M., and D. M. Crothers.** 1981. Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. Nucleic Acids Res. **9**:6505–6525.
7. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
8. **Grosschedl, R., and D. Baltimore.** 1985. Cell-type specificity of immunoglobulin gene expression is regulated by at least three DNA sequence elements. Cell **41**:885–897.
9. **Kelley, D. E., C. Coleclough, and R. P. Perry.** 1982. Functional significance and evolutionary development of the 5′-terminal regions of immunoglobulin variable-region genes. Cell **29**:681–689.
10. **Landolfi, N. F., J. D. Capra, and P. W. Tucker.** 1986. Interaction of cell type-specific nuclear proteins with immunoglobulin V_H promoter region sequences. Nature (London) **323**:548–551.
11. **Meyuhas, O., and R. P. Perry.** 1980. Construction and identification of cDNA clones for mouse ribosomal proteins: applications for the study of r-protein gene expression. Gene **10**: 113–129.
12. **Meyuhas, O., E. A. Thompson, Jr., and R. P. Perry.** 1987. Glucocorticoids selectively inhibit translation of ribosomal protein mRNAs in P1798 lymphosarcoma cells. Mol. Cell. Biol. **7**:2691–2699.
13. **Rosales, R., M. Vigneron, M. Macchi, I. Davidson, J. H. Xiao, and P. Chambon.** 1987. *In vitro* binding of cell-specific and ubiquitous nuclear proteins to the octamer motif of the SV40 enhancer and related motifs present in other promoters and enhancers. EMBO J. **6**:3015–3025.
14. **Singh, H., R. Sen, D. Baltimore, and P. A. Sharp.** 1986. A nuclear factor that binds to a conserved sequence motif in transcriptional control elements of immunoglobulin genes. Nature (London) **319**:154–158.
15. **Staudt, L. M., H. Singh, R. Sen, T. Wirth, P. A. Sharp, and D. Baltimore.** 1986. A lymphoid-specific protein binding to the octamer motif of immunoglobulin genes. Nature (London) **323**: 640–643.

16. **Xiao, J.-H., I. Davidson, D. Ferrandon, R. Rosales, M. Vigneron, M. Macchi, F. Ruffenach, and P. Chambon.** 1987. One cell-specific and three ubiquitous nuclear proteins bind *in vitro* to overlapping motifs in the domain B1 of the SV40 enhancer. EMBO J. **6**:3005–3013.
17. **Xiao, J.-H., I. Davidson, M. Macchi, R. Rosales, M. Vigneron, A. Staub, and P. Chambon.** 1987. *In vitro* binding of several cell-specific and ubiquitous nuclear proteins to the GT-I motif of the SV40 enhancer. Genes Dev. **1**:794–807.
18. **Yang, J.-Q., E. F. Remmers, and K. B. Marcu.** 1986. The first exon of the c-*myc* proto-oncogene contains a novel positive control element. EMBO J. **5**:3553–3562.