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Complete nucleotide sequence of a sea urchin actin gene

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ABSTRACT

We have determined the complete nucleotide sequence of a sea urchin actin gene, including the entire protein-coding sequence, introns and approximately 500 and 700 nucleotides adjacent to protein-coding-sequence on the 5' and 3' sides, respectively. This gene is split between codons 121 and 122 and within codon 204 by two introns which are 233 and 181 nucleotides in length, respectively. Comparison of the sequence of the two introns indicates a region of distant relatedness which covers about 25% of their lengths, suggesting that these sequences might have derived from a common ancestral sequence. The encoded amino acid sequence, which matches closely with that reported for actins from other species, is more cytoplasmic-like than muscle-like when compared to vertebrate actins. Analysis of the coding-flanking regions indicates the presence of sequences similar to those thought to be important for initiation of transcription and polyadenylation of mRNA. The location of these sequences and the size of an actin mRNA, transcribed from this or a very closely related gene, suggests that initiation occurs 347 nucleotides 5' of coding and polyadenylation approximately 515 nucleotides 3' of coding.

INTRODUCTION

Actin is a highly conserved protein, which is present in every eukaryotic tissue and cell type where it has been looked for (1). Since it is present in distantly related organisms from divergent branches of the phylogenetic tree, its gene must have been formed early in eukaryotic evolution. In addition, the high degree of conservation of its primary sequence must result from considerable selective constraints imposed due to its function(s) in the cell. Because the actin genes are ancient and highly conserved, an examination of their structure establishes an important framework in which to consider the origin and evolution of eukaryotic gene structure. More specifically, it is possible to compare the number and location of introns in these genes from eukaryotic species located anywhere on the phylogenetic tree and therefore construct a detailed evolutionary history of this important gene family. Also, since members of the actin

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gene family are known to be differentially expressed in various tissues, stages of development and cell types of several organisms (2-13), they are also an excellent set of genes in which to examine the relationship between gene structure and expression.

Here we report the complete nucleotide sequence of a sea urchin actin gene which is thought to be expressed in early embryogenesis (9,14). The sequence includes both introns and approximately 500 nucleotides of contiguous 5' and 700 nucleotides of contiguous 3' non-coding sequence. Analysis of this sequence demonstrates the location of the two introns and that the encoded protein is more cytoplasmic-like than muscle-like. Additionally, we find sequences in the 5' coding-adjacent region which correspond to the consensus TATA and $\begin{matrix}T\\C\end{matrix}$CAAT sequences and in the 3' region which resemble the consensus AATAAA sequence. The locations of these putative transcription initiation and polyadenylation associated sequences are appropriate to produce an actin mRNA of the expected size.

MATERIALS AND METHODS

DNA Sequencing

Nucleotide sequences were determined by the procedure of Maxam and Gilbert (16). Restriction enzyme digested DNA fragments were labeled either at the 5' end with [γ-^{32}P]ATP and polynucleotide kinase or at the 3' end with [α-^{32}P]cordycepin triphosphate and terminal transferase. The labeled fragments were cleaved by a secondary restriction enzyme digestion and the resulting fragments separated by polyacrylamide gel electrophoresis.

RESULTS AND DISCUSSION

Intron Locations and Structure

We previously reported that the recombinant plasmid, pSpG17, contains an actin-coding DNA fragment from the genome of the sea urchin, Strongylocentrotus purpuratus, and that the gene is split with at least one intron (15). Using the procedure of Maxam and Gilbert (16), we have now determined the sequence of 2749 of the approximately 3600 nucleotides of sea urchin DNA present in the plasmid. Figure 1 is a restriction map of the cloned sea urchin DNA which indicates the regions that have been sequenced. From this sequence it can be seen that the entire protein-coding portion of the gene lies approximately in the center of the cloned fragment with about 1 kilobase (kb) of non-coding sea urchin sequence to either side. The sequence itself and the encoded amino acids are displayed in Figure 2.

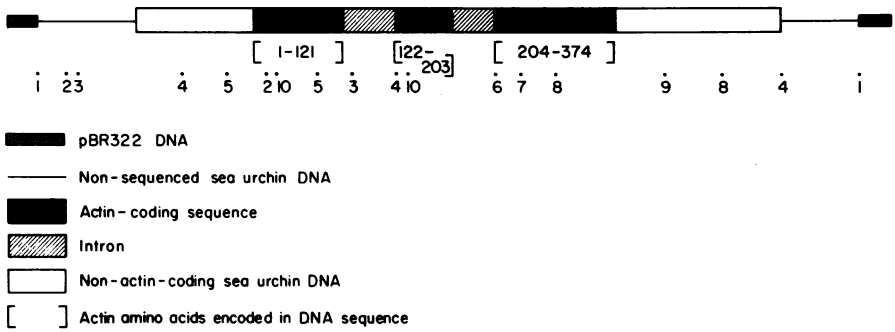
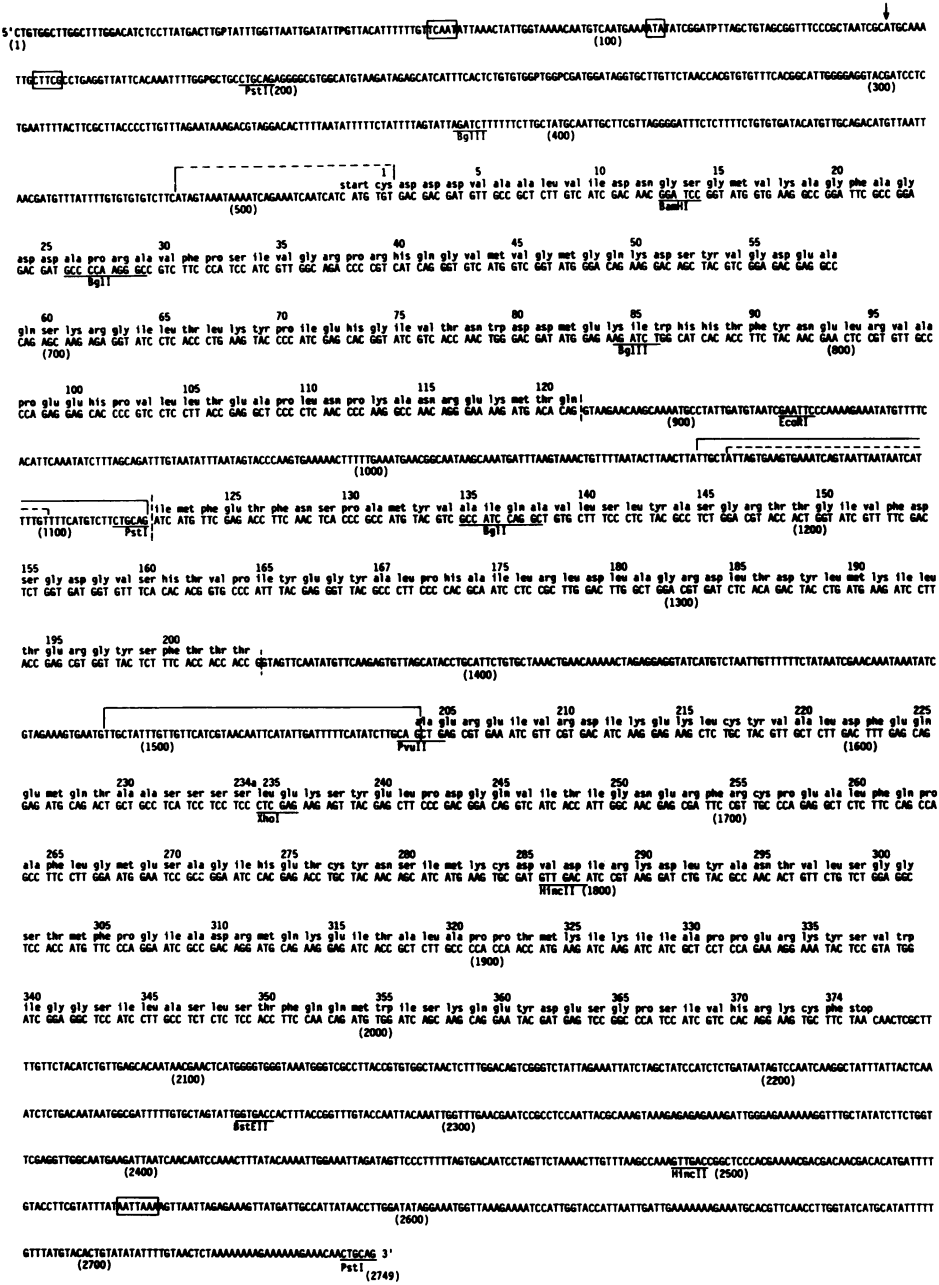


Figure 1. Map of the recombinant clone pSpG17 showing restriction enzyme sites and sequenced regions. Restriction enzyme sites are represented on the figure by the following numbers: 1, Hind III; 2, BamHI; 3, EcoRI; 4, PstI; 5, BglII; 6, PvuII; 7, XhoI; 8, HincII; 9, BstEII; 10, BglI. The sea urchin DNA has a total length of approximately 3.6 kb, and is not cut by the enzymes KpnI, SalI, XbaI or ClaI.

Comparison of the encoded amino acid sequence with that of known actins indicates that the gene is split in two locations, at amino acid coding positions 121/122 and 203/204. The lengths of the two introns are 233 and 181 nucleotides, respectively. In addition, the G+C content of the introns (27 and 31% respectively) differs strikingly from that of the protein-coding sequence, which is 55%. The sequences at the junctions of each intron and protein-coding sequence correspond closely with the consensus sequences derived by comparison of this region in a large number of eukaryotic genes (17,18). By application of the GT-AG rule (17) the splice points can be inferred (Figure 2). According to this analysis, the first intron splits the gene between codons 121 and 122, while the second splits the gene within codon 204.

Comparison of the precise locations of the introns in this gene to those in actin genes from species on divergent branches of the phylogenetic tree indicates a considerable diversity of intron locations among these highly conserved genes. None of the actin-gene-intron locations found in Drosophila, a protostome [codons 13 and 307 (19-21)], yeast, a fungus [codon 4 (22,23)] or Dictyostelium, a slime mold [none (24)] correspond to those seen in this sea urchin (deuterostome) gene. Such diversity of intron location might not previously have been recognized in other genes because it has not been possible to compare the structure of protein-coding genes from such distantly related species. Similarities of intron location are seen, how-



ever, in actin genes within the deuterostome branch of animal evolution. The intron positions reported here (i.e., codons 121/122 and 203/204) are found in a rat α-actin gene (codon 204) (25), a rat β-actin gene (codon 121) (25), and a chicken α-actin gene (codon 204, Ordahl and Kuncio, personal communication). Two other sea urchin actin gene intron locations at codons 41/42 and 267/268 in a different gene (which also contains introns at codons 121/122 and 203/204, Crain and Cooper, unpublished) are also seen in the rat (25) and chicken (Ordahl and Kuncio, personal communication) α-actin genes. In light of these observations it is possible that, within the deuterostome branch of animal evolution, the actin genes might have evolved from a single ancestral gene with multiple introns which were subsequently deleted, as suggested by Gilbert (26). On the other hand, lack of coincidence of intron locations in actin genes from other branches of the phylogenetic tree suggests that loss of introns during evolution from a single primordial gene may not be a sufficient explanation for the structure of modern actin genes. On the contrary, it seems possible, if not likely, that new introns have been inserted into these genes at various times during their evolution. In the case of the sea urchin genes it also seems that duplication events have given rise to multiple similar genes. This is suggested by the following set of observations. (i) There are at least 7 two-intron-containing sea urchin actin genes and at least 5 of these are closely linked to another two-intron gene (9). (ii) These two-intron genes are at most 2% divergent from one another (9). (iii) At least one additional sea urchin gene type exists which contains four introns, does not seem to be linked to any two-

Figure 2. Nucleotide sequence of the sea urchin actin gene contained in the recombinant plasmid pSpG17 and the amino acid sequence of the encoded protein. The nucleotides are numbered from 1 to 2749 in parenthesis below the sequence at every hundred nucleotides, with the first digit directly under the nucleotide with that number (N = unspecified nucleotide, P = purine, Y = pyrimidine). Regions which resemble consensus sequences found in the 5' and 3' regions of eukaryotic genes are boxed and are located at the following positions: CAAT sequence, 71-75; ATA sequence, 108-110; putative ribosome binding site, 158-162; AAT(T)AAA sequence, 2552-2558. The most likely point of initiation of transcription is at nucleotide 148 and is indicated by ↓. The inferred splice points at the coding-intron borders are indicated by ¦. The encoded amino acids, which are shown above the appropriate codons, are numbered at every fifth position starting immediately after the initiator methionine codon and ending with amino acid number 374 which is immediately followed by a stop codon. The encoded actin sequence begins with a cysteine residue as has been inferred for all six Drosophila actins from the gene sequences (20,21). The restriction enzyme recognition sites shown in Figure 1 are underlined. Two sets of homologous sequences are indicated by ⌜---⌝ (490-524; 1063-1100) and ⌜⌝ (1058-1116; 1493-1545) over the sequences.

intron genes and is 10% divergent from the pSpG17 gene over 40% of its protein-coding sequence (Crain and Cooper, unpublished). Thus it appears that at some point in sea urchin evolution and possibly as early as the time of the echinoderm radiation there existed a small number of two actin gene types (two- and four-introns) and that at least one of these (the two-intron type) was subsequently duplicated resulting in multiple linked copies of this actin gene type. It has been suggested that such gene duplication events may be important in establishing new genomic regulatory modules necessary for the evolution of new biological structures (27). Examination of the actin gene types and their copy number and linkage patterns in other echinoderms would define how recently this gene duplication event has occurred.

To determine whether the sequences of the two introns are related, we searched for homologies using a computer program (SEQ) described by Brutlag et al. (28). This computer assisted comparison of the intron sequences revealed a region of 67.7% homology at the 3' end of each intron, which extends across 60 and 53 nucleotides in the first and second introns, respectively (shown in Figure 2). The match (which contained a maximum loopout of either DNA strand of 2 nucleotides) was considered to be statistically highly significant (i.e., Expectation $\leq$ 0.01). This similarity between the two introns, which spans 25.8% ($\frac{60}{233}$) and 29.3% ($\frac{53}{181}$) of their respective lengths, suggests that, at least in this region, they are distantly related to each other. The possibility is thus raised that they derive from a common ancestral sequence. If this were the case it would suggest that the two introns were formed, at least partially, as a result of a common event; which could have been the integration of a common sequence.

Comparison of the intron sequences to the coding-adjacent sequence further revealed a 5' sequence with 75.7% homology with a region at the 3' end of the first intron (this match contained loopouts of only 1 nucleotide in either DNA strand and was statistically significant; i.e., Expectation $\leq$ 0.05). This homology, which extends 6 nucleotides into the beginning of protein-coding sequence, also falls within the region, described above, which the introns have in common (shown in Figure 2). It thus appears that there are sequences present to the 5' side of each block of protein-coding sequence which are distantly related. The extent of difference between these regions suggests that they are not involved in a function requiring a high degree of sequence specificity. It seems more likely that they are the remnants of a common ancestral sequence, as discussed above. Recognition of

related sequences such as these within a gene, may be helpful in elucidating the evolutionary events which resulted in the modern gene.

5' and 3' Protein-Coding-Flanking Regions Contain Putative Transcription Initiation and Polyadenylation Signals

Analysis of the sequence which flanks the actin-coding region of this gene reveals the presence of sequences which are characteristic of many eukaryotic genes and are thought to be important for transcription and translation. The location of these sequences suggests approximate positions for messenger RNA initiation and polyadenylation. In the 5' flanking sequence there are five AT-rich regions which contain the tri-nucleotide ATA and therefore resemble the consensus TATA sequence reported to lie approximately 30 nucleotides 5' of the mRNA capping site of all cellular eukaryotic genes transcribed by RNA polymerase II (29-31). One of these (ATA at positions 108-110, Figure 2) begins 33 nucleotides to the 3' side of the sequence TCAAT (positions 71-75, Figure 2), which matches with the consensus $\begin{matrix}C\\T\end{matrix}$CAAT sequence found to be located approximately -70 to -80 nucleotides upstream from the mRNA capping site in many eukaryotic genes (30-32). The relationship of these two sequences to each other is characteristic of the 5' sequence of many eukaryotic genes and suggests that transcription should be initiated approximately 30 nucleotides 3' of the ATA sequence. Additionally, the sequence CAT (positions 147-149, Figure 2), which begins 37 nucleotides 3' of the ATA conforms to the properties of an mRNA start site; that is, an A surrounded by pyrimidines (31). From this analysis and the observation that most eukaryotic messages are initiated with an A (31), we conclude that the most likely point of mRNA initiation is at the A found at position 148. Furthermore, initiation at this site would give a mature mRNA of the expected size (see below).

The only sequence which has been recognized as being consistently present to the 3' side of protein-coding sequence among eukaryotic cellular genes is AATAAA or a related sequence AATTAAA found approximately 20 nucleotides 5' of the polyadenylation site. We find the sequence AATTAAA at positions 2552-2559 (beginning 489 nucleotides 3' of the TAA stop codon). Comparison of the sequence of this gene with that of the cDNA clone SpG2 [a plasmid constructed by cloning RNA·DNA hybrids after reverse transcription of poly(A) containing RNA from sea urchin gastrula; (33,9)] shows 97.8% homology between the two. Thus SpG2 probably derives from a transcript of the pSpG17 gene. While it is possible that SpG2 derives from a different but closely related gene, the sequence differences are most likely due to

sequencing errors and/or polymorphisms between individuals. The 3' end of SpG2 is at nucleotide 2498 of the pSpG17 sequence, indicating that the messenger RNA extends at least 435 nucleotides beyond coding. Since no AATAAA sequence is present in the 3' untranslated portion of SpG2, it was previously suggested that reverse transcriptase synthesis may not have initiated at the 3' end of the message (9). The presence of the AATTAAA sequence an additional 54 nucleotides beyond the end of SpG2 supports this hypothesis and suggests that the polyadenylation site might be located approximately 20 nucleotides beyond this sequence.

Assuming these inferred initiation and polyadenylation sites, a mature message transcribed from this gene would consist of approximately 374 nucleotides of 5' non-coding sequence and 515 nucleotides of 3' non-coding sequence. The predicted total message length would thus be 2100-2200 nucleotides [374+515+1125 (protein-coding) + 100-200 (poly A)]. Since an mRNA of approximately 2.2 kb in sea urchin embryos is transcribed from this or a closely related gene (9,14), it is possible that the initiation and polyadenylation sites are at the approximate locations defined here.

Relationship of this Gene and its Encoded Protein to Other Actin Genes and Proteins

Comparison of the amino acid sequences of various mammalian actins indicates that there are substitutions at specific locations which appear to be characteristic of muscle and cytoplasmic actins (34,35). The question of how crucial these residues are for the proposed different functions of these actin variants may ultimately be answered by comparison of the sequences of actins specifically expressed in various tissues from many different species. The actin encoded in pSpG17 does not strictly conform to either mammalian type; that is, it contains muscle-like, cytoplasmic-like and previously unreported residues at the putative diagnostic positions. Of the 22 most characteristic sites, the sea urchin protein contains 16 cytoplasmic-like substitutions (6, 10, 16, 76, 103, 162, 176, 201, 225, 259, 266, 286, 296, 298, 357, 364), three muscle-like substitutions (17, 271, 278), one previously unreported substitution (153) and one substitution found in *Physarum* and yeast and not in mammalian actins (5). Although this actin does not fall entirely into either category, it is considerably more cytoplasmic-like than muscle-like. It has been noted that actins from lower eukaryotes [*Dictyostelium discoideum* (36); *Physarum polycephalum* (37)] and insects [*Drosophila melanogaster* (20,21)] have greater similarity to vertebrate cytoplasmic actins than to vertebrate muscle actins. Whether sea urchins

contain a gene which encodes a more typical muscle actin will be determined by sequencing other cloned genes.

To further examine the relationship between this sea urchin gene and encoded protein and other actins and actin genes, we compared the DNA and encoded protein sequence to other reported protein and complete actin gene sequences (Table 1). As has been previously pointed out, the degree of conservation of the actin amino acid sequence across large phylogenetic distances is quite remarkable, with the largest difference noted here being only 11.5% between the yeast and the sea urchin proteins. The sea urchin actin is most similar to mammalian cytoplasmic actin (4.3% amino acid sequence difference), the actins from the lower eukaryotes, Dictyostelium discoideum (5.6%) and Physarum polycephalum (4.8%) and two Drosophila actins (6.4 and 5.6%). It is possible that all of these actins are cytoplasmic, with similar functions and thus similar selective constraints. On the other hand the differences between sea urchin and rabbit skeletal muscle (8.3%) and yeast (11.5%) are noticeably greater. Since sea urchins are more closely related to mammals (both are deuterostomes) than to Drosophila (a protostome) and the sea urchin actin is more similar to Drosophila actins than rabbit skeletal muscle actin, it is likely that the vertebrate muscle actins are evolving under somewhat different selective constraints than the cytoplasmic species. Furthermore, since the Drosophila actin gene, 79B, which is more similar to mammalian cytoplasmic actins than muscle actin, is thought to encode a larval muscle actin (11), the rather distinctive vertebrate muscle actins may have evolved after the branch which gave rise to the protostomes and deuterostomes as suggested by Fyrberg et al., (20). The yeast actin, which shows a greater difference from this sea urchin actin

Table 1. Sequence Comparison of the Sea Urchin Actin Gene and Encoded Protein with Other Actin Genes and Proteins.

Actin Gene or Protein		Difference in Amino Acid Sequence Compared to Protein Encoded in pSpG17[a]	Difference in Protein-Coding Nucleotide Sequence Compared to pSpG17[a]
Rabbit skeletal muscle	(39)	31/375 = 8.3%	
Mammalian cytoplasmic	(35)	16/375 = 4.3%	
Drosophila (79B)	(21)	24/375 = 6.4%	197/1125 = 17.5%
Drosophila (88F)	(21)	21/375 = 5.6%	194/1125 = 17.2%
Dictyostelium discoideum	(37)	21/375 = 5.6%	
Physarum polycephalum	(38)	18/375 = 4.8%	
Yeast	(22,23)	43/375 = 11.5%	275/1125 = 23.6%

[a]In the cases of the yeast and mammalian cytoplasmic actins, which do not contain an amino acid corresponding to position number 1, this was calculated as a difference of one amino acid or three nucleotides.

than the actins from the other reported species (11.5%) is puzzling in the context of the above considerations. Since yeast has no muscle, it is reasonable to conclude that its actin is non-muscle in function, yet it is not closely related to the cytoplasmic-like actins. The yeast actins, therefore, may be somewhat unique in their function and their evolution.

The divergence of the nucleotide sequence between the sea urchin gene and the other three reported complete actin gene sequences corresponds to phylogenetic relatedness to the extent that the gene from the most distantly related organism, yeast, shows a greater difference (23.6%) than either of the Drosophila genes (17.2 and 17.5%). The Drosophila genes, which encode proteins that differ from the sea urchin actin by 24 (79B) and 21 (88F) amino acids, each show a similar nucleotide sequence divergence compared to the sea urchin gene (17.5 and 17.2%, respectively). Further breakdown of the differences between the genes indicates that the percent silent substitutions (those which do not change the amino acid sequence) are 14.5 (Drosophila, 79B) and 14.7 (Drosophila, 88F) and 18.3 (yeast). These differences approach the maximum difference possible with no change in amino acid sequence [approximately 23% (38)] and thus suggest no unusual selection on the nucleotide sequence itself.

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REFERENCES

1. Pollard, T. D. and Weihing, R. R. (1974) Crit. Rev. Biochem. 2, No. 1, 1-65.
2. Garrels, J. J. and Gibson, W. (1976) Cell 9, 793-805.

3. Storti, R. and Rich, A. (1976) Proc. Natl. Acad. Sci. USA 73, 2346-2350.
4. Whalen, R. F., Butler-Browne, G. S. and Gross, F. (1976) Proc. Natl. Acad. Sci. USA 73, 2018-2022.
5. Storti, R., Horovitch, S., Scott, M., Rich, A. and Pardue, M. (1978) Cell 13, 589-598.
6. Devlin, R. B. and Emerson, C. P. (1978) Cell 13, 599-611.
7. Crain, W. R., Durica, D. S. and Van Doren, K. (1981) Mol. Cell. Biol. 1, 711-720.
8. McKeown, M. and Firtel, R. A. (1981) Cell 24, 799-807.
9. Scheller, R. H., McAllister, L. B., Crain, W. R., Durica, D. S., Posakony, J. W., Thomas, T. L., Britten, R. J. and Davidson, E. H. (1981) Mol. Cell. Biol. 7, 609-628.
10. Shani, M., Zevin-Sonkin, D., Saxel, O., Carmon, Y., Katcoff, D., Nudel, U. and Yaffe, D. (1981). Dev. Biol. 86, 483-492.
11. Zulauf, E., Sanchez, F., Tobin, S. L., Rdest, U. and McCarthy, B. J. (1981) Nature 292, 556-558.
12. Durica, D. S. and Crain, W. R. (1982) Dev. Biol., in press.
13. Ernst, S. G., Bushman, F. D. and Crain, W. R. (1982) submitted.
14. Crain, W. R., Durica, D. S., Cooper, A. D., Van Doren, K. and Bushman, F. D. (1982) Cold Spring Harbor Laboratory Monograph "Muscle Development", in press.
15. Durica, D. S., Schloss, J. A. and Crain, W. R. (1980) Proc. Natl. Acad. Sci. USA 77, 5683-5687.
16. Maxam, A. M. and Gilbert, W. (1980) in Methods in Enzymology, 65, L. Grossman and K. Moldave, eds. (New York: Academic Press), pp. 499-560.
17. Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. and Chambon, P. (1978) Proc. Natl. Acad. Sci. USA 75, 4853-4857.
18. Lerner, M. R., Boyle, J. A., Mount, S. M., Wolin, S. L. and Steitz, J. A. (1980) Nature 283, 220-224.
19. Fyrberg, E. A., Kindle, K. L., Davidson, N. and Sodja, A. (1980) Cell 19, 365-378.
20. Fyrberg, E. A., Bond, B. J., Hershey, N. D., Mixter, K. S. and Davidson, N. (1981) Cell 24, 107-116.
21. Sanchez, F., Tobin, S. L., Rdest, U., Zulauf, E. and McCarthy, B. J. (1982) submitted.
22. Gallwitz, D. and Sures, I. (1980) Proc. Natl. Acad. Sci. USA 77, 2546-2550.
23. Ng, R. and Abelson, J. (1980) Proc. Natl. Acad. Sci. USA 77, 3912-3916.
24. Firtel, R. A., Timm, R., Kimmel, A. R. and McKeown, M. (1979) Proc. Natl. Acad. Sci. USA 76, 6206-6210.
25. Nudel, U., Katcoff, D., Zakut, R., Shani, M., Carmon, Y., Finer, M., Czosnek, H., Ginsburg, I. and Yaffee, D. (1982) submitted.
26. Gilbert, W. (1979) in Eukaryotic Gene Regulation, R. Axel, T. Maniatis and C. F. Fox, eds. (New York: Academic Press) pp. 1-22.
27. Davidson, E. H., Thomas, T. L., Scheller, R. H. and Britten, R. J. (1982) in Genome Evolution, G. Dover and R. B. Flavell, eds. (Academic Press, London), in press.
28. Brutlag, D. L., Clayton, J., Friedland, P. and Kedes, L. H. (1982) Nucl. Acids Res. 10, 279-294.
29. Goldberg, M. (1979) Ph.D. thesis, Stanford University, Stanford, CA.
30. Efstratiadis, A., Posakony, J. W., Maniatis, T., Lawn, R. M., O'Connell, C., Spritz, R. A., DeRiel, J. K., Forget, B. G., Weissman, S. M., Slightom, J. L., Blechl, A. E., Smithies, O., Baralle, F. E., Shoulders, C. C., Proudfoot, N. J. (1980) Cell 21, 653-668.
31. Breathnach, R. and Chambon, P. (1981) Ann. Rev. Biochem. 50, 349-383.

32. Benoist, C., O'Hare, K., Breathnach, R. and Chambon, P. (1980) Nucleic Acids Res. 8, 127-142.
33. Lasky, L. A., Lev, Z., Xin, J. H., Britten, R. J. and Davidson, E. H. (1980) Proc. Natl. Acad. Sci. USA 77, 5317-5321.
34. Vandekerckhove, J. and Weber, K. (1978) Proc. Natl. Acad. Sci. USA 75, 1106-1110.
35. Vandekerckhove, J. and Weber, K. (1979) Differentiation 14, 123-133.
36. Vandekerckhove, J. and Weber, K. (1980) Nature 284, 475-477.
37. Vandekerckhove, J. and Weber, K. (1978) Nature 276, 720-721.
38. Kimura, M. (1968) Genet. Res. Camb. 11, 247-269.
39. Elzinga, M. and Lu, R. C. (1976) in Contractile Systems in Non-Muscle Tissues, eds. Perry, S. V., Margreth, A. and Edelstein, R. S. (Elsevier) North Holland, Amsterdam), pp. 29-37.