Expression of the genes coding for the skeletal muscle and cardiac actins in the heart

Y. Mayer, H. Czosnek, P.E. Zeelon¹, D. Yaffe and U. Nudel

Department of Cell Biology, The Weizmann Institute of Science, Rehovot 76100, and
¹Biotechnology General, Inc., Nes Ziona, Israel

Received 27 September 1983; Revised and Accepted 13 December 1983

ABSTRACT

Several types of evidence indicate that the gene coding for the skeletal muscle actin is expressed in the rat heart: 1) A recombinant plasmid containing an insert with a nucleotide sequence identical to that of the homologous region of skeletal muscle actin gene was isolated from a cDNA library prepared on rat cardiac mRNA template. 2) Using specific probes it was found that the hearts of newborn rats contain a significant amount of skeletal muscle actin mRNA. The quantity of this mRNA in the heart decreases during development. 3) The skeletal muscle actin gene is DNAase I sensitive in nuclei from rat heart tissue.

A plasmid containing a cDNA insert homologous to a part of the cardiac actin mRNA was isolated and sequenced. It was found that in spite of the great similarity between the amino acid sequence of the skeletal muscle and cardiac actins, the nucleotide sequences of the two mRNAs are considerably divergent. There is only limited sequence homology between the 3' untranslated regions of the two mRNAs. However, there is an extensive sequence homology between the 3' untranslated regions of the rat and human cardiac mRNAs, suggesting a functional role for this region of the gene or mRNA.

INTRODUCTION

The actins constitute a family of highly conserved proteins, found in all eukaryotes examined so far. Studies at the protein level have identified six different actins in mammals: two nonmuscle β - and γ -actins, which were found in most or all cell types, and four muscle actins which were considered tissue-specific: the skeletal muscle actin, the cardiac muscle actin and two smooth muscle actins (1-5).

Recently, it has been shown that an mRNA coding for a protein very similar or identical to the cardiac actin is present in fetal and neonatal mouse skeletal muscle. The amount of this mRNA decreases rapidly during development (6). Here we report on the isolation, from a cDNA library prepared on rat cardiac mRNA template, of a plasmid containing a DNA insert with a sequence identical to that of the homologous parts of the skeletal muscle actin gene, and on the presence of the skeletal muscle actin mRNA in the heart

tissue. The amount of the skeletal muscle actin mRNA seems to be developmentally regulated: it is present in a significant quantity in the heart of newborn rats, but its amount decreases subsequently.

Comparison of the nucleotide sequence of a cardiac actin cDNA clone with that of the rat skeletal muscle actin gene reveals that the 3' untranslated regions of the two mRNAs show only limited sequence homology. In contrast, comparison between the 3' untranslated regions of the rat and human cardiac actin mRNAs reveals a very great conservation of the nucleotide sequence.

MATERIALS AND METHODS

Construction of the cDNA plasmids pC18 and pS2

Single- and double-stranded cDNA was synthesized on a 1-month old rat cardiac mRNA template, using reverse transcriptase. The double-stranded cDNA was inserted into the PstI site of pBR322, using the C-G tailing technique. Recombinant plasmids were screened for actin DNA sequences with the ³²P-labelled 3.7 kb DNA fragment from the recombinant bacteriophage Act15. This fragment contains most of the rat skeletal muscle actin gene (7). Positive clones were further analyzed by DNA sequencing.

Preparation of plasmids and DNA probes

Plasmid DNA was prepared by the alkaline extraction method (8) and purified by centrifugation to equilibrium in cesium chloride/ethidium bromide gradients. Restricted DNA fragments were purified by electrophoresis on agarose gels or on acrylamide gels, electroelution, and chromatography on DEAE-cellulose. The DNA fragments were labelled by nick translation (9) to a specific activity of ca. 4×10^8 cpm/ug.

Preparation, electrophoresis and blot hybridization of RNA

Total cellular RNA was prepared from various tissues by the lithium chloride/urea extraction method (10), electrophoresed in formaldehyde/agarose gels, and blotted to nitrocellulose membrane (11). Hybridization was done in 50% formamide, as described by Alwine et al. (12). After the hybridization the RNA blots were washed in 0.1 xSSC (1 xSSC = 0.15 M sodium chloride, 0.015M trisodium citrate) at 50°C for 2 h, followed by washing in 0.1 xSSC at 61°C for 30 min. Great care was taken during dissection of the heart to remove as much as possible the major blood vessels and other nonmuscle tissues.

S1 endonuclease mapping

The appropriate DNA fragment was end-labelled by filling in with reverse transcriptase, strand separated, and the non-sense strand was incubated with

total cardiac RNA treated with 500 units of endonuclease S1 (Miles) for 30 min at 37°C and electrophoresed on a 6% polyacrylamide/urea sequencing gel (13), using labelled pBR322 DNA fragments as size markers. The gels were then fluorographed.

DNA sequencing

DNA fragments were end-labelled either by filling in with reverse transcriptase or by the exchange reaction using Klenow's enzyme. The sequencing was done by the chemical cleavage technique described by Maxam and Gilbert (14).

Calculation of sequence homology

The percentage of divergence in silent sites was calculated according to Perler et al. (15; no correction for multiple changes was made). The homology between 3'-untranslated regions was calculated by aligning the two sequences to be compared in such a way as to obtain maximal homology and minimal single base deletions. Mismatches, including gaps, were counted. The divergence was calculated as 100x the ratio between the number of mismatches and the length of DNA. (Each gap was scored as one mismatch and therefore was counted as one nucleotide in the overall count of the length of the DNA.)

Determination of DNAase I sensitivity of actin genes

One-month old Wistar male rats were sacrificed, the hearts quickly excized and rinsed in ice-cold PBS. The tissue was minced with scissors and the blood was washed off. The minced tissue was homogenized using a Teflon-glass homogenizer (20 strokes) in 10 mM Tris pH 7.8, 1 mM EDTA, 3 mM CaCl₂, 10 mM KCl (TECK; Ref. 16). Nonidet P-40 was added to the homogenate (to 0.3%) and the homogenate was filtered through four layers of fine cheesecloth. The nuclei were collected by centrifugation at 1000 xg for 3 min, resuspended in TECK, and washed two additional times by centrifugation and suspension. The preparation of nuclei was finally suspended in the DNAase I buffer described by Wu et al. (17), pelleted, and resuspended in this buffer at a concentration of 1 mg/ml of nucleic acids (20 OD₂₆₀ units in 0.5 N NaOH, 1 M NaCl = 1 mg/ml).

Aliquots of isolated nuclei (1 mg/ml) were incubated for 10 min at 37°C with increasing amounts of DNAase I (Worthington), as shown in Fig. 4. The reaction was stopped with 0.3% SDS, 12 mM EDTA, 50 ug/ml proteinase K. After an overnight incubation at 37°C the DNA was extracted according to Bellard et al. (18).

The DNA samples were digested with EcoR1, electrophoresed and transferred to nitrocellulose papers, as described by Carmon et al. (19). Filters were prehybridized and then hybridized with nick-translated probes specific for the cardiac muscle actin gene (plasmid pC18), the skeletal muscle actin gene

(insert of plasmid p749; Ref. 21) and an immunoglobulin constant region (C_{κ}) gene (insert of plasmid B1; Ref. 19). After hybridization, the filters were washed at 70°C in 0.1 xSSC for 45 min and exposed to X-ray films. The fluorograms were scanned with a Beckmann DU-8 spectrophotometer, and the relative intensities of bands were determined as described in the legend to Fig. 4.

RESULTS

A. Isolation from a cardiac cDNA library of recombinant plasmids containing DNA sequences coding for skeletal muscle and cardiac actins

A cDNA library was prepared using polyadenylated RNA template isolated from 1-month old rat hearts. 2.6×10^4 colonies of the library were screened with the 3.7 kb EcoRI DNA fragment of the phage Act15 which contains most of the structural gene of the skeletal muscle actin (7). Three clones which hybridized with this probe were isolated. The isolated recombinant plasmids were hybridized to Southern blots of EcoRI digested rat spleen DNA. The blots were washed at high stringency (0.1 SSC, 50 min, 73°C) and exposed to X-ray film. Two plasmids (pC17 and pC18) hybridized to a 2.0 kb DNA fragment. We have shown earlier that the rat skeletal muscle actin gene is located on a 5.7 kb EcoRI DNA fragment and that, under these hybridization and washing conditions, plasmid p749 (which contains a cDNA insert specifying the C terminal half of the skeletal muscle actin) hybridized specifically to a single EcoRI fragment of 5.7 kb (21).

Sequencing of the insert of pS2 showed that it contains the codons specifying the C terminal 103 amino acids (272-374) of the skeletal muscle actin and 100 nucleotides of the 3' untranslated region. The nucleotide sequence was identical to that of the homologous regions of the skeletal muscle actin gene.

The insert of one of the two plasmids which hybridized specifically to a 2.0 kb EcoRI DNA fragment, pC18, was also sequenced. It has 279 nucleotides specifying the codons of the 93 C terminal amino acids (282-374) of a muscle actin, plus 163 nucleotides of the 3' untranslated region and a sequence of 18 adenylic acid residues at the 3' end. A polyadenylation signal sequence AATAAA is located 20 nucleotides 5' to the polyA tail. The insert contains codons for two amino acids which are different in bovine cardiac and skeletal muscle actins (the amino acid sequence of the rat cardiac actin has not yet been determined). At position 298 there is Leu in cardiac actin and Met in skeletal muscle actin. At position 357 there is Ser in cardiac actin and Thr in skeletal muscle actin (5). As shown in Fig. 1, the codons at positions 298 and 357 of pC18 specify the cardiac actin. The codons at positions 286, 296 and

364 differentiate between the muscle actins and the cytoplasmic actins (5). The insert of pC18 does not contain codons which allow for the distinction between smooth and cardiac muscle actins. However, using pC18 as a probe, we isolated a genomic DNA clone containing the complete rat cardiac actin gene. Partial DNA sequencing showed identity between the insert of pC18 and the homologous regions of the isolated gene; the sequenced region contains also the codons for the amino acids specifying the cardiac actin (data not shown).

B. The 3' untranslated region of the rat cardiac actin mRNA diverged greatly from that of the rat skeletal muscle actin but is very similar to that of the human cardiac actin mRNA

Comparison of the nucleotide sequence of pC18 with that of the rat skeletal muscle actin gene shows that in spite of the great similarity of the two proteins (1.1% amino acid replacements), the nucleotide sequences of the two genes has diverged considerably. The 3' untranslated region of the cardiac actin mRNA is 75 nucleotides shorter, and only limited sequence homology between the 3' untranslated regions of the two mRNAs is detected: two regions 25 and 15 nucleotides long are considerably conserved (85% and 75% homology, respectively; Fig. 1b). In particular, a short stretch, 10 nucleotides long, located 17 nucleotides downstream from the translation termination codon, has been conserved in the rat, human and chick skeletal muscle actin genes and in the rat and human cardiac actin genes (7, 22-24). The coding regions show a homology of 85% (58% homology in the silent sites).

On the other hand, comparison of the sequence of pC18 with that of the human cardiac actin gene (24) shows that the sequences of most of the 3' untranslated region are highly conserved. The homology between the 3' halves of these regions is over 92%. The 5' halves of the 3' untranslated regions are more diverged and contain several short nonhomologous sequences (Fig. 1).

In the coding regions the homology is over 92% (77% homology in the silent sites).

The 3' untranslated region contains several direct and inverted repeated sequences.

C. The presence of skeletal muscle actin mRNA in the heart

We have previously reported that plasmid p106 which contains the sequences of the 3' untranslated region of skeletal muscle actin mRNA, hybridized with cardiac mRNA. On the basis of these results we suggested the existence of some sequence homology between the 3' untranslated regions of the cardiac and skeletal muscle mRNA (20). The finding that there is no extensive sequence homology between the two 3' untranslated regions, and the isolation from a

Weber (5); h, the nucleotide sequence of the homologous regions of human cardiac actin gene (24). The alignment of the 3' untranslated region was made to obtain maximal homology. Identical nucleotides are marked by vertical dashes. Sequences which share high homology with sequences in the 3' untranslated region of the skeletal muscle actin gene are marked with horizontal dashed lines.

(b) Schematic presentation of sequence homologies in the 3' regions between rat and human cardiac actin genes and rat skeletal muscle and cardiac actin genes

Heavy solid bars, coding regions. Thin bars, 3' untranslated regions. Thin dashed line connects regions that show sequence homology and the percentage of homology between these regions is indicated. The homology in the silent sites in the coding region is given in parentheses. Empty bars, no significant homology was found.

cardiac cDNA library of the plasmid pS2 containing skeletal muscle actin DNA sequence, suggest that the observed hybridization of p106 with cardiac mRNA was due to the presence of skeletal muscle actin mRNA in the cardiac tissue. To further investigate this question we tested the hybridization of cloned cDNA probes containing sequences of the 3' untranslated regions of either the skeletal muscle or the cardiac actin mRNAs, with RNA extracted from several tissues. As can be seen from Fig. 2, in the newborn rat hearts there is a significant amount of RNA sequences which hybridize with the skeletal muscle actin cDNA probe. The relative amount of these sequences is smaller in RNA prepared from hearts of older animals.

The probe prepared from pC18 (homologous to the 3' untranslated region of the cardiac actin mRNA) hybridizes with RNA isolated from the heart as well as with RNA prepared from skeletal muscle of young rats (Fig. 2A) and from differentiated cultures of the myogenic cell line L8, but not with RNA from proliferating myoblasts or from rat brain (not shown). The presence of cardiac-like actin mRNA sequence in mouse myogenic cultures and in neonatal skeletal muscle was reported recently (6).

The amount of skeletal muscle actin mRNA sequences in RNA isolated from newborn hearts is about 10% of that in the leg muscle RNA. Within 80 days of development its amount in the heart decreases by a factor of 12 and increases in leg muscle by a factor of 2.3. The cardiac actin mRNA in RNA from leg muscle of newborn rats is about 13% of that in the heart muscle RNA. During 80 days of development it decreases by a factor of 130 and increases in heart muscle by a factor of 3.4.

The presence of skeletal muscle actin mRNA in cardiac tissue was further verified by S1 endonuclease digestion of RNA-DNA hybrids. A DNA fragment 465 nucleotides long, extending from the BamHI site in exon 6 of the skeletal muscle actin gene (Fig. 3f) to the EcoRI site of Act15 (7) was isolated and

FIGURE 2. Blot hybridization of RNA from skeletal and cardiac muscle to actin cDNA specific probes

RNA was prepared from skeletal and cardiac muscle of rats of the indicated age. Total RNA (40 ug/slot) was electrophoresed on formamide agarose gels and blotted to nitrocellulose filter. Filter A was hybridized with a fragment of pC18 homologous to the 3' untranslated region of the rat cardiac actin mRNA. Filter B was hybridized with the insert of p106 homologous to the 3' untranslated region of the skeletal muscle actin mRNA. After hybridization the filters were washed as described in Materials and Methods and fluorographed. (A slightly stronger hybridization of skeletal muscle RNA from 30-day old rats with pC18, compared with RNA from 15-day old skeletal muscle, was observed in several independent experiments.)

end-labelled with reverse transcriptase. The two DNA strands were separated and the nonsense strand was hybridized with total RNA preparations from rat skeletal muscle, cardiac muscle and brain. After hybridization and digestion with S1 endonuclease, the samples were electrophoresed on a polyacrylamide/urea sequencing gel. As can be seen in Fig. 3, both the skeletal muscle RNA and the cardiac RNA protected a DNA fragment 162 nucleotides long (the protected fragment extends from the BamHI site to the end of the sixth exon of the skeletal muscle actin gene; 7). The amount of protected DNA fragment was much smaller when cardiac RNA from 30- and 80-day old rats was used for hybridization (not shown). No protection was observed with brain RNA. These

FIGURE 3. S1 endonuclease analysis of the presence of skeletal muscle actin mRNA in rat heart

RNA preparations from rat brain (c), cardiac muscle (d), and skeletal muscle (e) were hybridized to the single-stranded end-labelled 465-nucleotide long BamHI-EcoRI DNA fragment of plasmid pAct15.2 (7), treated with endonuclease S1, electrophoresed on acrylamide urea sequencing gel and fluorographed (see Materials and Methods). a, Size markers (HinfI digest of pBR322); b, the untreated probe; f, a schematic presentation of the rat skeletal muscle actin gene, the end-labelled probe and the protected fragment.

results confirm the conclusion that the skeletal muscle actin gene is expressed in the heart tissue, and that the amount of the skeletal muscle mRNA decreases during development.

D. DNAse I sensitivity of the skeletal muscle actin gene in heart nuclei

The presence of a small amount of skeletal muscle actin mRNA in the cardiac tissue may be the result of either a low activity of this gene in many cardiac cells or a high activity of the gene in a small population and perhaps a specific type of cell present in the heart.

It has been shown that active genes are organized in the chromatin in a conformation which renders them preferentially sensitive to digestion with nucleolytic enzymes (19, 25, 26). We showed previously that the skeletal muscle actin gene is preferentially sensitive to DNAse I in nuclei isolated from differentiated muscle cells, which synthesize skeletal muscle actin, but not in nuclei from proliferating mononucleated myoblasts and from the brain

FIGURE 4. DNAase I sensitivity of the cardiac muscle and skeletal muscle actin genes in isolated heart nuclei

Southern blots of EcoRI digested DNA from DNAase I-treated nuclei were hybridized with radiolabelled probes specific for the cardiac muscle actin gene, the skeletal muscle actin gene and an immunoglobulin gene (see Materials and Methods). At high stringency wash, each probe produced a single radioactive band (2.0, 5.7 and 6.8 kb, respectively). The relative intensities of the autoradiographic bands for each DNAase I concentration was determined by densitometric scans. The DNAase I sensitivity index was determined by dividing the intensity of the bands corresponding to the fragments containing the cardiac and the skeletal muscle actin genes in each track by the value of the immunoglobulin gene band in the same track. The values are normalized; the ratios obtained for DNA from untreated nuclei equal 1. □, cardiac muscle actin gene; △, skeletal muscle actin gene.

(19). We therefore tested the DNAase I sensitivity of the skeletal muscle actin gene in nuclei isolated from the heart. It was assumed that the relative sensitivity of the skeletal muscle actin gene would indicate whether this gene was active in the majority of the nuclei or in a small fraction. As can be seen in Fig. 4, in nuclei isolated from 1-month old rat cardiac tissue, the 5.7 kb EcoRI DNA fragment which contains the skeletal muscle actin gene is as sensitive as the 2 kb EcoRI DNA fragment containing sequences of the cardiac actin gene. These results suggest that the skeletal muscle actin gene is in an active state in a significant proportion of the nuclei isolated from the heart tissue. Had it been active only in a small proportion of the nuclei, it would have been hard to notice the DNAase I sensitivity of this gene on the background of hybridizable DNA from nuclei in which the gene was inactive, and not preferentially sensitive to DNAase I.

DISCUSSION

The present investigation provided several types of evidence showing that

the gene coding for an actin which had hitherto been considered to be specific for the skeletal muscle is expressed also in the rat heart. These data also indicate that the same skeletal muscle actin gene is active in both tissues.

The concentration of skeletal muscle actin mRNA in cardiac RNA of 1-day old rats is approximately 10% of that in the leg muscle RNA. It drops to approximately 0.5% in cardiac mRNA of 1-month old rats. Although we did not determine the presence of skeletal muscle actin in the heart, there is no reason to think that the skeletal muscle actin mRNA is not translated in this tissue. The failure to detect previously the skeletal muscle actin in the heart can be attributed to the fact that there are only 4 amino acid replacements between the skeletal muscle and cardiac actins, and that the two proteins are indistinguishable by two-dimensional isoelectric focussing SDS gel electrophoresis. Impurities of less than 5% are undetectable by amino acid sequencing. Since adult heart was used as a source of actin for the amino acid sequencing, the level of the skeletal muscle actin in these preparations was most probably below 5%.

It is impossible as yet to assess the biological significance of the presence of the 2 muscle actins in the heart tissue and the changes in their relative amounts during development. An inverse situation was observed in the developing skeletal muscle: A significant amount of a cardiac-like actin mRNA was found in embryonic and neonatal skeletal muscle. The relative amount of this mRNA decreased rapidly during development. There are also tissue- and stage-specific isoforms of myosin heavy and light chains, the synthesis of which is developmentally regulated (27-29). The exchange in isoforms of myosin heavy and light chains in cardiac muscle was not studied as extensively as that in skeletal muscle. Nevertheless, it is evident that the expression of various isoforms of myosin heavy and light chains is developmentally regulated also in heart muscle, and switches between embryonic and adult isoforms were observed (30-32).

Several possible explanations for the apparently developmentally regulated expression of the two actin genes in cardiac and skeletal muscles should be considered: 1) Since the muscles of embryos and adults operate in different physiological environments, they may require different sets of contractile proteins, each fitting better to a specific stage of development. Although no functional difference has been found so far between the different muscle actins, they may behave differently in the intact muscle cells. The identity of amino acid sequences in all actins from adult skeletal muscle examined so far in mammals and in the chicken and the identity of bovine and human cardiac

actins may indicate that the existence of 4 amino acid differences between the skeletal muscle and cardiac actins has a physiological role. 2) It is possible that the mechanism of control of gene expression during development involves the activation of a new set of genes for each stage of development. Accordingly, each isoform is a member of a set of proteins whose genes are coordinatively activated in a certain muscle tissue during a specific stage of development. It should be noted that the skeletal muscle and the cardiac actin genes are located on different chromosomes (33). Therefore, the co-expression of the two genes during early development is not due to their proximity in a single chromatin domain. 3) The sequential expression of the isoforms of some of the contractile proteins had perhaps, in the past, a regulatory or a physiological role that has been lost during evolution and the ontogenetic expression of the genes reflects a phylogenetic remnant. 4) During the period of fast growth of the muscle tissue, a single gene is not sufficient for the synthesis of enough actin. This need is transiently compensated for by the activation of an extra actin gene(s). Additional information on both the regulation of expression and the physiology of the contractile apparatus may help to understand the significance of the mode of expression of the 2 actin isoproteins in the skeletal and cardiac muscle.

In spite of the great similarity of the cardiac and skeletal muscle actins and their expression in the same tissues, as well as the identity in the position of the introns in the coding region of the 2 genes (7, 24), comparison of the nucleotide sequence reveals a considerable degree of divergence. The 3' untranslated regions show very limited sequence homology, and the sequenced parts of the coding region have a homology of 58% in the silent sites. The two genes are also located on different chromosomes (33). These data indicate a relatively ancient separation of the two genes; the preservation of almost identical amino acid sequences of the two actins indicates a very high selective pressure to conserve this sequence.

On the other hand, the comparison between the nucleotide sequence of rat and human cardiac actin genes reveals a very high degree of homology. Two large segments which constitute most of the 3' untranslated regions show 92% and 85% homology (Fig. 1b). (The overall homology between the two 3' untranslated regions is 82%.) It is of interest that the silent sites in the coding region which is included in pC18 have diverged to a greater extent (77% homology). Furthermore, we have recently sequenced the region coding for amino acids 1-150 of the rat cardiac actin gene. Comparison with the human cardiac actin gene shows that the divergence in the silent sites is 30% and that in

the sequenced introns is much greater. Considering the fact that in a gene coding for a very conserved protein the coding region is subjected only to point mutations, and that there are certain constraints on the type of replacements in the silent sites (due to the nonrandom pattern of codon usage), the higher conservation of the sequences in the 3' untranslated region is rather puzzling. It should be noted also that large parts of the 3' untranslated regions of rat and human cytoplasmic β -actin genes show a high degree of homology (34, 35). It seems, therefore, that there was a selective pressure to conserve large parts of the 3' region of the rat and human cardiac actin gene (and perhaps of the β -actin gene). Such a conservation may indicate the presence of a functionally important structure (or sequence) in this region of the cardiac actin gene or in its transcripts. Comparison of other rat/human pairs of genes (the insulin genes (36, 37), the growth hormone gene (38, 39), and the prolactin genes (40, 41)) reveals a great divergence in the 3' untranslated regions (37-45% divergence). However, an almost identical sequence (96% homology), 27 nucleotides long, is found in the 3' end of the 3' untranslated regions of both human and rat growth hormone genes.

ACKNOWLEDGMENTS

The authors thank Ms. Z. Levy, Ms. S. Neuman and Ms. O. Saxel for their excellent technical assistance. The work was supported by the Muscular Dystrophy Association, Inc., USA, by the US-Israel Binational Science Foundation, Jerusalem, and by NIH grant R01 GM 22767. U. Nudel is the incumbent of the A. and E. Blum Career Development Chair in Cancer Research.

REFERENCES

1. Storti, R.V. and Rich, A. (1976) *Proc. Natl. Acad. Sci. USA* 73, 2346-2350
2. Whalen, R.G., Butler-Browne, G.S. and Gros, F. (1976) *Proc. Natl. Acad. Sci. USA* 73, 2018-2022
3. Garrels, J. and Gibson, W. (1976) *Cell* 9, 793-805
4. Elzinga, M. and Lu, R. (1979) in "Contractile Systems in Nonmuscle Tissues" (eds. Perry, S.V., Margreth, A. and Adelstein, R.), North-Holland, Amsterdam, p. 29
5. Vanderkerckhove, J. and Weber, K. (1979) *Differentiation* 14, 124-133
6. Minty, J.A., Alonso, S., Caravatti, M. and Buckingham, M.E. (1982) *Cell* 30, 185-192
7. Zakut, R., Shani, M., Givol, D., Neuman, S., Yaffe, D. and Nudel, U. (1982) *Nature* 298, 857-859
8. Birnboim, H.C. and Doly, J. (1979) *Nucleic Acids Res.* 7, 1513-1523
9. Maniatis, T., Jeffrey, A. and Kleid, D.G. (1975) *Proc. Natl. Acad. Sci. USA* 72, 1184-1188
10. Auffray, C., Nageotte, R., Chambraud, B., Rougeon, F. (1980) *Nucleic Acids Res.* 8, 1231-1241

11. Goldberg, D.A. (1980) *Proc. Natl. Acad. Sci. USA* 77, 5794-5798
12. Alwine, J.C., Kemp, D.J. and Stark, R.G. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5350-5354
13. Berk, A.J. and Sharp, P.A. (1977) *Cell* 12, 721-732
14. Maxam, A.M. and Gilbert, W.R. (1977) *Proc. Natl. Acad. Sci. USA* 74, 560-564
15. Perler, F., Efstratiadis, A., Lomedico, P., Gilbert, W., Kolodner, R. and Dodgson, J. (1980) *Cell* 20, 555-566
16. Mellon, I. and Bohrjee, J.S. (1982) *Exp. Cell Res.* 137, 141-154
17. Wu, C., Bingham, P.M., Livak, K.J., Homgren, R. and Elgin, S.C.R. (1979) *Cell* 16, 797-806
18. Bellard, M., Gannon, F. and Chambon, P. (1978) *Cold Spring Harbor Symp. Quant. Biol.* 42, 779-791
19. Carmon, Y., Czosnek, H., Nudel, U., Shani, M. and Yaffe, D. (1982) *Nucleic Acids Res.* 10, 3085-3097
20. Shani, M., Nudel, U., Zevin-Sonkin, D., Zakut, R., Givol, D., Katcoff, D., Carmon, Y., Rieter, J., Frischauf, A.-M. and Yaffe, D. (1981) *Nucleic Acids Res.* 9, 579-589
21. Nudel, U., Katcoff, D., Zakut, R., Shani, M., Carmon, Y., Finer, M., Czosnek, H., Ginsburg, I. and Yaffe, D. (1982) *Proc. Natl. Acad. Sci. USA* 79, 2763-2767
22. Fornwald, J.A., Kuncio, G., Peng, I. and Ordahl, C.P. (1982) *Nucleic Acids Res.* 10, 3861-3876
23. Ordahl, C.P. and Cooper, T.A. (1983) *Nature* 303, 348-349
24. Hamada, H., Petrino, M.G. and Kakunaga, T. (1982) *Proc. Natl. Acad. Sci. USA* 79, 5901-5905
25. Weintraub, H. and Groudine, M. (1976) *Science* 193, 848-856
26. Garrel, A. and Axel, R. (1976) *Proc. Natl. Acad. Sci. USA* 73, 3966-3970
27. Whalen, R.G., Sell, S.M., Butler-Browne, G.S., Schwartz, K., Bouveret, P. and Pinset, I. (1981) *Nature* 292, 805-809
28. Whalen, R.G., Sell, S.M., Eriksson, A. and Thornell, L.E. (1982) *Dev. Biol.* 91, 478-484
29. Whalen, R.G., Butler-Browne, G.S. and Gros, F. (1978) *J. Mol. Biol.* 126, 415-431
30. Lompre, A.M., Mercadier, J.J., Wisniewsky, C., Bouveret, C., Pantaloni, C., J'Albis, A. and Schwartz, K. (1981) *Dev. Biol.* 84, 286-290
31. Whalen, R.G. and Sell, S.M. (1980) *Nature* 286, 731-733
32. Chizzonite, R.A., Everett, W., Clark, W.A., Jakovcic, S., Rabinowitz, M. and Zak, R. (1982) *J. Biol. Chem.* 257, 2056-2065
33. Czosnek, H., Nudel, U., Mayer, Y., Barker, P.E., Pravtcheva, D.D., Ruddle, F.H. and Yaffe, D. (1983) *EMBO J.* 2, in press
34. Nudel, U., Zakut, R., Shani, M., Neuman, S., Levy, Z. and Yaffe, D. (1983) *Nucleic Acids Res.* 11, 1759-1771
35. Hanukoglu, I., Tanese, N. and Fuchs, E. (1983) *J. Mol. Biol.* 163, 673-678
36. Lomedico, P., Rosenthal, N., Efstratiadis, A., Gilbert, W., Kolodner, R. and Tizard, R. (1979) *Cell* 18, 545-558
37. Bell, G.I., Pictet, R.L., Rutter, W.J., Cordell, B., Tischer, E. and Goodman, H.M. (1980) *Nature* 284, 26-32
38. Denote, F.M., Moore, D.D. and Goodman, H.M. (1981) *Nucleic Acids Res.* 9, 3719-3730
39. Page, G.S., Smith, S. and Goodman, H.M. (1981) *Nucleic Acids Res.* 9, 2087-2104
40. Cooke, N.E., Coit, D., Shnie, J., Baxter, J.D. and Martial, J.A. (1981) *J. Biol. Chem.* 256, 4007-4016
41. Cooke, N.E., Coit, D., Weiner, R.I., Baxter, J.D. and Martial, J.A. (1980) *J. Biol. Chem.* 255, 6502-6510