Vol. 167, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
February 28, 1990 Pages 323-330

THE TWO mRNA FORMS FOR THE TYPE I_α REGULATORY SUBUNIT OF cAMP-DEPENDENT PROTEIN KINASE FROM HUMAN TESTIS ARE DUE TO THE USE OF DIFFERENT POLYADENYLATION SITE SIGNALS

Mårten Sandberg[1,2], Bjørn Skålhegg[1,3] and Tore Jahnsen[1,2]

[1]Laboratory for Gene Technology, Institute of Pathology, Rikshopitalet, University of Oslo, N-0027 Oslo 1, Norway

[2]Institute of Medical Biochemistry, University of Oslo, P.O. Box 1112, Blindern, N-0317 Oslo 3, Norway

[3]Institute of General and Immunological Rheumatology, Rikshospitalet, University of Oslo, N-0027 Oslo 1, Norway

Received January 2, 1990

SUMMARY: Using a cDNA probe for the type I_α regulatory subunit, two mRNA species (1.5 and 3.0 kb in length) were detected in human testis. From a human testis cDNA-library a 3.0 kb clone, containing the entire reading frame of the protein, was isolated. Comparison of the nucleotide sequence of this clone to the sequence of a 1.5 kb cDNA clone earlier reported, showed that the longer clone was identical to the shorter but extended another 1.5 kb in the 3' end. Sequencing data together with Northern blot analysis indicated that the two mRNA species for human type I_α regulatory subunit were generated from the same gene by the use of different polyadenylation site signals.

The cAMP-dependent protein kinase holoenzyme (EC 2.7.1.37) is made up of two regulatory (R) and two catalytic (C) subunits and undergoes dissociation into free fully active C subunits and an R_2-4cAMP complex when activated by cAMP (1,2). The free C subunits catalyze phosphorylation of their specific substrate proteins, thereby activating or inactivating them (3).

For a long time the cAMP-dependent protein kinase has been divided into type I and type II based upon their elution from DEAE-cellulose (4). It was shown that the R subunits were different in the two types, and for that reason the two different

The abbreviations used are: cDNA, complementary DNA; RI, regulatory subunit of the type I cAMP-dependent protein kinase; RII, regulatory subunit of the type II cAMP-dependent protein kinase; SDS, sodium dodecyl sulphate; SSC, standard saline citrate (0.15 M NaCl, 15 mM sodium citrate, pH 7.0).

0006-291X/90 $1.50

R subunits were called RI and RII, respectively. It has been shown recently that there exist two different isoforms for both RI, RI_α (5,6) and RI_β (7), and for RII, RII_α (8,9) and RII_β (10,11). Two different isoforms for the catalytic subunits, C_α (12,13) and C_β (13-15) have also been isolated from several species. In addition, a third catalytic isoform, C_γ, has been detected in human testis (16).

Northern blot analysis has demonstrated the existence of more than one mRNA species for several of the subunits of the cAMP-dependent protein kinase. This has been the case for RI_α, RII_α and RII_β (6,17,8,18,10). Until now, it has remained unclear as to whether the two mRNA species detected by the RI_α cDNA probe are due to crosshybridization between transcripts from two distinct but closely related genes, or to alternative splicing, or to the use of different polyadenylation site signals of pre mRNAs from a single gene. We report here the molecular cloning of a full length 3.0 kb cDNA for human RI_α and results from Northern analysis that together indicate that the two mRNA species are caused by the use of different polyadenylation site signals.

EXPERIMENTAL PROCEDURES

cDNA-cloning: Phage plaques from a human testis lambda gt11 library (Clontech, Palo Alto, Ca (Cat#HL 1010) were transferred to nitrocellulose filters, denatured, baked, and prehybridized at 68 °C for 2-20 hrs in 6 X SSC, 2 X Denhardt's solution (19). Hybridization was carried out for at least 6 hrs using the same buffer and temperature. The probe used was a radioactively labelled 0.6 kb Pst I fragment from the coding region of mouse RI_α (kindly provided by Dr. G. S. McKnight, Seattle, WA). The filters were washed twice in 1 X SSC, 0.5 % SDS for two hrs at 68 °C before they were dried and autoradiographed.

Nucleotide sequencing: The dideoxy chain termination method (20) using the modified T7 DNA polymerase (21) ("Sequenase" from United States Biochemical Corporation, Cleveland, Ohio, U.S.A. (Cat#70700)) was used.

Computer analysis: Nucleotide sequence data was analysed using the program package from the University of Wisconsin Genetics Computer Group (22).

Northern blot analysis: Total RNA was isolated by the guanidine isothiocyanate method of Chirgwin et al (23) and was then denatured in 50 % (v/v) formamide, 6.0 % (v/v) formaldehyde followed by 15 min on ice, and resolved on a 1.5 % agarose gel containing 6.7 % formaldehyde, 20 mM sodium phosphate, pH 7.0. The gel was blotted onto a nylon membrane, and baked at 80 °C for 1 hour. The membrane was prehybridized in 5 X Denhardt's solution, 5 X SSC, 50 mM sodium phosphate buffer, pH 6.5, 0.1 % (w/v) SDS, 250 µg/ml salmon sperm DNA, and 50 % (v/v) formamide at 42 °C, and hybridized using similar conditions in a buffer containing the radioactively labelled probe. The membrane was washed four times in 2 X SSC, 0.1 % (w/v) SDS at room temperature for 5-10 minutes, followed by two washes using 0.1 X SSC, 0.1 % (w/v)

SDS at 50 °C. The membrane was autoradiographed at -70 °C for variable periods of time.

RESULTS AND DISCUSSION

In an earlier study we described the isolation of several positive clones from a human testis cDNA library using a mouse RI_α cDNA as a probe (6). One of the positive clones, clone A, was characterized and shown to represent a 1476 nucleotide long cDNA encoding a full-length human RI_α protein. When clone A was used in Northern blot analysis, two mRNA species, 1.5 and 3.0 kb in length, were detected in human testis with the 1.5 kb form predominating. In the other tissues examined only the 3.0 kb mRNA species was detected (data not shown). The clone A cDNA contained a poly A tail and most probably represented the 1.5 kb mRNA species in testis.

Because of the existence of the 3.0 kb mRNA species, a further characterization of the other isolated clones were undertaken in an attempt to reveal what the 3.0 kb mRNA species represented. Several genetic mechanisms have been described that can explain the existence of two or more mRNA species being detected by the same probe. Firstly, the two mRNA species can represent transcripts from two distinct, but closely related genes, which are so similar that the probe detects both. Secondly, the two mRNA species may represent distinct transcripts from

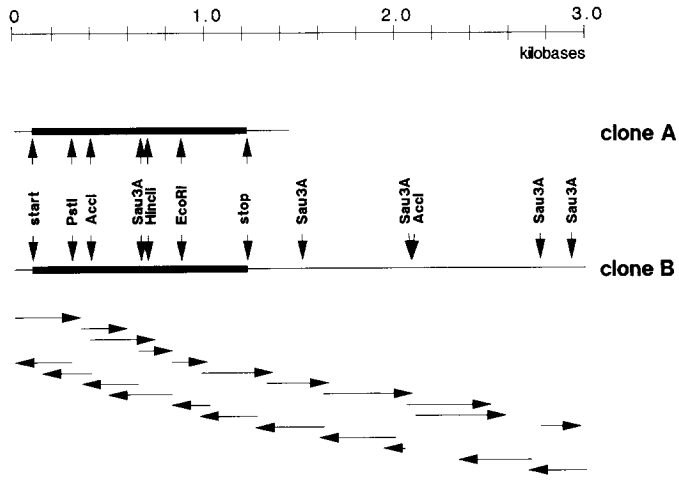

Fig. 1. Restriction map of the 1.5 and the 3.0 kb cDNAs for the human testis RI_α. Restriction sites are indicated at approximate positions within the map. The black bars indicate the reading frame. The arrows show which of the subclones of the cDNA have been sequenced.

```
-87  GCTGGGAGCAAAGCGCTGAGGGAGCTCGGTACGCCGCCGCCTCGCACCCGCAGCCTCGCGCCCGCCGCCGCCCGTCCCCAGAGAACCATGGAGTCTGGCAGT  15
                                                                                    MetGluSerGlySer

 16  ACCGCCGCCAGTGAGGAGGCACGCAGCCTTCGAGAATGTGAGCTCTACGTCCAGAAGCATAACATTCAAGCACTGCTCAAAGATTCTATTGTGCAGTTGTGC  117
     ThrAlaAlaSerGluGluAlaArgSerLeuArgGluCysGluLeuTyrValGlnLysHisAsnIleGlnAlaLeuLeuLysAspSerIleValGlnLeuCys

118  ACTGCTCGACCTGAGAGACCCATGGCATTCCTCAGGGAATACTTTGAGAGGTTGGAGAAGGAGGAGGCAAAACAGATTCAGAATCTGCAGAAAGCAGGCACT  219
     ThrAlaArgProGluArgProMetAlaPheLeuArgGluTyrPheGluArgLeuGluLysGluGluAlaLysGlnIleGlnAsnLeuGlnLysAlaGlyThr

220  CGTACAGACTCAAGGGAGGATGAGATTTCTCCTCCTCCACCCAACCCAGTGGTTAAAGGTAGGAGGCGACGAGGTGCTATCAGCGCTGAGGTCTACACGGAG  321
     ArgThrAspSerArgGluAspGluIleSerProProProProAsnProValValLysGlyArgArgArgArgGlyAlaIleSerAlaGluValTyrThrGlu

322  GAAGATGCGGCATCCTATGTTAGAAAGGTTATACCAAAAGATTACAAGACAATGGCCGCTTTAGCCAAAGCCATTGAAAAGAATGTGCTGTTTTCACATCTT  423
     GluAspAlaAlaSerTyrValArgLysValIleProLysAspTyrLysThrMetAlaAlaLeuAlaLysAlaIleGluLysAsnValLeuPheSerHisLeu

424  GATGATAATGAGAGAAGTGATATTTTTGATGCCATGTTTTCGGTCTCCTTTATCGCAGGAGAGACTGTGATTCAGCAAGGTGATGAAGGGGATAACTTCTAT  525
     AspAspAsnGluArgSerAspIlePheAspAlaMetPheSerValSerPheIleAlaGlyGluThrValIleGlnGlnGlyAspGluGlyAspAsnPheTyr

526  GTGATTGATCAAGGAGAGACGGATGTCTATGTTAACAATGAATGGGCAACCAGTGTTGGGGAAGGAGGGAGCTTTGGAGAACTTGCTTTGATTTATGGAACA  627
     ValIleAspGlnGlyGluThrAspValTyrValAsnAsnGluTrpAlaThrSerValGlyGluGlySerPheGlyGluLeuAlaLeuIleTyrGlyThr

628  CCGAGAGCAGCCACTGTCAAAGCAAAGACAAATGTGAAATTGTGGGGCATCGACCGAGACAGCTATAGAAGAATCCTCATGGGAAGCACACTGAGAAAGCGG  729
     ProArgAlaAlaThrValLysAlaLysThrAsnValLysLeuTrpGlyIleAspArgAspSerTyrArgArgIleLeuMetGlySerThrLeuArgLysArg

730  AAGATGTATGAGGAATTCCTTAGTAAAGTCTCTATTTTAGAGTCTCTGGACAAGTGGGAACGTCTTACGGTAGCTGATGCATTGGAACCAGTGCAGTTTGAA  831
     LysMetTyrGluGluPheLeuSerLysValSerIleLeuGluSerLeuAspLysTrpGluArgLeuThrValAlaAspAlaLeuGluProValGlnPheGlu

832  GATGGGCAGAAGATTGTGGTGCAGGGAGAACCAGGGGATGAGTTCTTCATTATTTTAGAGGGGTCAGCTGCTGTGCTACAACGTCGGTCAGAAAATGAAGAG  933
     AspGlyGlnLysIleValValGlnGlyGluProGlyAspGluPhePheIleIleLeuGluGlySerAlaAlaValLeuGlnArgArgSerGluAsnGluGlu

934  TTTGTTGAAGTGGGAAGATTGGGGCCTTCTGATTATTTTGGTGAAATTGCACTACTGATGAATCGTCCTCGTGCTGCCACAGTTGTTGCTCGTGGCCCCTTG  1035
     PheValGluValGlyArgLeuGlyProSerAspTyrPheGlyGluIleAlaLeuLeuMetAsnArgProArgAlaAlaThrValValAlaArgGlyProLeu

1036 AAGTGCGTTAAGCTGGACCGACCTAGATTTGAACGTGTTCTTGGCCCATGCTCAGACATCCTCAAACGAAACATCCAGCAGTACAACAGTTTTGTGTCACTG  1137
     LysCysValLysLeuAspArgProArgPheGluArgValLeuGlyProCysSerAspIleLeuLysArgAsnIleGlnGlnTyrAsnSerPheValSerLeu

1138 TCTGTCTGAAATCTGCCTCCTGTGCCTCCCTTTTCTCCTCTCCCCAATCCATGCTTCACTCATGCAAACTGCTTTATTTTCCCTACTTGCAGCGCCAAGTGG  1239
     SerValEnd

1240 CCACTGGCATCGCAGCTTCCTGTCTGTTTATATATTGAAAGTTGCTTTTATTGCACCATTTTCAATTTGGAGCATTAACTAAATGCTCATACACAGTTAAAT  1341
1342 AAATAGAAAGAGTTCTATGGAGACTTTGCTGTTACTGCTTCTCTTTGTGCAGTGTTAGTATTCACCCTGGGCAGTGAGTGCCATGCTTTTTGGTGAGGGCAG  1443
1444 ATCCCAGCACCTATTGAATTACCATAGAGTAATGATGTAACAGTGCAAGATTTTTTTTTTAAGTGACATAATTGTCCAGTTATAAGCGTATTTAGACTGTGG  1545
1546 CCATATATGCTGTATTTCTTTGTAGAATAAATGGTTTCTCATTAAACTCTAAAGATTAGGGAAAATGGATATAGAAAATCTTAGTATAGTAGAAAGACATCT  1647
1648 GCCTGTAATTAAACTAGTTTAAGGGTGGAAAAATGCCCATTTTTGCTAATTATCAATGGGATATGATTGGTTCAGTTTTTTTTTTCCAGAGTTGTTGTTTGCC  1749
1750 AAGCTAATCTGCCTGGTTTTATTTATATCTTGTTATTAATGTTTCTTCTCCAATTCTGAAATACTTTTGAGTATGGCTATCTATACCTGCCTTTTAAGTTTG  1851
1852 AAACTAACTCATAGATTGCAAATATTGGTTAGTATTTAACTACATCTGCCTCGGCTCACAAATTCCGATTAGACCTTTATCCAGCTAGTGCCAAATAATTGA  1953
1954 TCAGATGCTGAATTGAGAATAAGAATTTGAGGTCTACATTCTTGGTTGTTAATTTAGAGCGTTTGGTTAAAGTATGTCCTTCAGCTGACTCCAGTATAATCT  2055
2056 CCTCTGCTCATTAAACTGATTCCAGGAGATTGGATTTGCTGTGACTAGATACAGATGGAGCAAATGTCCTAACAGAGAAATAGAGGTGATGCTGCTAAAGGG  2157
2158 AGAAATGCCAGGCGGACAAAGTTCAGTGTCGGGAATTTTCCCCGTGACATTCACTGGGGCATGAGATTTTGGAAGAAGTTTTTTACTTTGGTTTAGTCTTTT  2259
2260 TTTCCTTCCTTTTTATTCAGCTAGAATTTCTGGTGGGTTGATGGTAGGGTATAATGTGTCTGTGTTGCTTCAAATTGGTCTGAAAGGCTATCCTGCTGAAAG  2361
2362 TCCTGCTTTCCTATCTAGCATTTATTCCTCTGGCAAACTTTCTTTCTTTTCTTTTTTAAAGTAAACTTGTGTATTGAGTCTTAACTGTATTTCAGTATTTT  2463
2464 CCAGCCTTATGTGTTACATTATTCCAATGATACCCAACAGTTTATTTTTATTATTTTTTTAAACAAAATTTCACAGTTCTGTAATGTAGGCACTTTTATTTT  2565
2566 CATTGTGATTTATATATAAGGTAATGTAGGGTTATATTTGGGAGTGACTGCAAGCATTTTTCCATCTGTGTGCAACTAACTGACTCTGTTATTGATCCCTTC  2667
2668 TCCTGCCCTTTCCCAGGTAATTTAAATTGGTCATGGTAGATTTTTTTCATAGATTTGAAAAACTTTTAGGTTGTTACCAAGTATGAAGTATAAATCTGGGGA  2769
2770 AGAGGTTTTATTTACATTTTAGGGTGGGTAAGAAAGCCACCTTGTTACAAATTTTTAATTTCCAAAATAATCTATATTAAATGAGGGTTTCTGATCTGTAC  2871
2872 TTTGTGTTTAGCTACCTTTTTATATTTAAAAAATTAAAAATGAAAATTATGTTCTTACAAGCTTAAAGCTTGATTTGATCT  2952
```

Fig. 2. Nucleotide and amino acid sequence of the 3.0 kb cDNA for human testis RI_α. Nucleotides are numbered starting with 1 at the first nucleotide in the coding region. A total of seven polyadenylation site signals are underlined. Nucleotide 1362 (underlined) represents the last nucleotide present in both the shorter and the longer human RI_α cDNAs.

the same gene because of alternative splicing (reviewed in 24-26) or be the result of the use of different polyadenylation site signals. This latter mechanism has been shown to be responsible for the existence of several mRNA species for the rat RII_β subunit of the cAMP-dependent protein kinase (10).

Sequencing of one of the clones (Fig. 1), clone B, revealed a cDNA insert 3038 nucleotides in length, containing the entire coding region of the RI_α subunit (Fig. 2). Clone B contained 87 nucleotides 5' of the initiator ATG compared to 103 nucleotides in clone A. The reading frames, 1143 nucleotides in length, were

identical in the two clones, and encoded the 381 amino acid long RI_α subunit. The lengths of the 3' nontranslated region, however, differed substantially in the two clones. In clone A this region was 216 nucleotides long including a short polyA tail (13 A's). The corresponding region of clone B consisted of 1806 nucleotides, of which the first 203 were identical to the 3' nontranslated region of clone A with the exception of one nucleotide. Clone A had a polyadenylation site signal appropriately located close to the polyA tail. A total of seven polyadenylation site

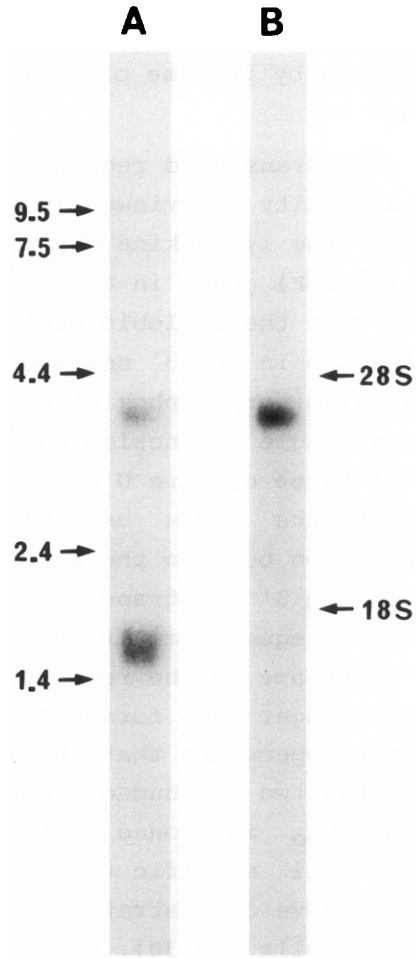

Fig. 3. Northern blot analysis. Lane A shows human testis RNA hybridized with the 5' EcoRI fragment, while lane B shows the same filter hybridized with the 3' AccI/EcoRI fragment. The hybridizing bands were estimated to be 1.5 and 3.0 kb in length by comparison with mRNA standards of 9.5, 7.5, 4.4, 2.4, and 1.4 kb (Bethesda Research Laboratories).

signals were found in clone B, and in contrast to clone A, clone B contained no polyA tail.

It seemed likely that the clone B cDNA represented the longer mRNA species found in testis and other human tissues. To verify this idea clone B was digested with Acc I to give a 1.0 kb 3' Acc I/Eco RI fragment (corresponding to nucleotides 1987-2952 in the sequence) that was unique for the longer cDNA (Fig. 1). This fragment was radioactively labelled and used as a probe in Northern analysis of testis RNA. Only the 3.0 kb mRNA species was detected. On the other hand, when a 0.8 kb 5' Eco RI fragment (corresponding to nucleotides -87 to 742) was used as a probe both mRNA species were detected. These data together with our sequencing information indicated that the 1.5 and 3.0 kb mRNA for human RI_α were generated by the use of different polyadenylation site signals (Fig.3).

Sequences in 3'nontranslated regions have been shown to be important for mRNA stability (reviewed in 27). Insertion of AU rich sequences from the lymphokine granulocyte-monocyte colony stimulating factor (GM-CSF) gene in the 3' nontranslated region of the ß-globin gene make the ß-globin mRNA highly unstable (28). The most striking feature in the 3' nontranslated regions of the GM-CSF gene and several other lymphokine and proto-oncogene mRNAs is the ocurrence of a single adenosine nucleotide followed by a polyuridine tract of three or more U's. It is not known why this sequence motif makes the mRNAs unstable, but one possible explanation is interaction between the AU rich sequence and small nuclear RNPs (28). In the 3' nontranslated region of the longer mRNA for human RIα the sequence motif AUUU appears a total of 34 times. Only two of these are in the region that is common between the shorter and the longer mRNA forms. Based on this consideration, it is tempting to speculate that the 1.5 kb mRNA species is the more stable of the two in human testis. In rat testis the shorter mRNA form for RI_α is found only in germ cells (17) suggesting a similar cell specific expression in human testis. Several investigators have demonstrated a characteristic translational delay in germ cells (29,30), mouse protamine mRNAs being one example (31). The selection of stable mRNA species in germ cells may be essential to ensure certain levels of mRNA for translation at late spermatid stages after cessation of transcription in order to maintain an adequate level of proteins necessary for late spermatid differentiation. Also for the RII_α

and RII_β subunits of the human cAMP-dependent protein kinase, smaller-sized mRNA have been detected in the germ cells (17,18).

ACKNOWLEDGMENTS: We gratefully appreciate the technical assistance of Gro Knutsen, Inger Ronander and Anne Keiserud. This work was supported by the Norwegian Cancer Society, Norwegian Research Council for Science and the Humanities (NAVF), Torsteds grant, Nordic Insulin Foundation and Anders Jahres Foundation for the Promotion of Science.

REFERENCES

1. Corbin, J.D., Sugden, P.H., West, L., Flockhart, D.A., Lincon, T.M. and McCarthy, D. (1978) J. Biol. Chem. 253, 3997-4002
2. Builder, S.E., Beavo, J.A. and Krebs, E.G. (1980) J. Biol. Chem. 255, 3514-3519
3. Krebs, E. G. (1989) JAMA 262 (13), 1815-1818
4. Corbin, J.D., Keely, S.L. and Park, S.R. (1975) J. Biol. Chem. 250, 218-225
5. Kuno, T., Ono, Y., Hirai, M., Hashimoto, S., Shuntoh, H. and Tanaka, C. (1987) Biochem. Biophys. Res. Comm. 146(2), 878-883
6. Sandberg, M., Taskén, K., Øyen, O., Hansson, V. and Jahnsen, T. (1987) Biochem. Biophys. Res. Comm. 149(3), 939-945
7. Clegg, C. H., Cadd, G. G. and McKnight, G. S. (1988) Proc. Natl. Acad. Sci. U.S.A. 85, 3703-3707
8. Scott, J. D., Glaccum, M. B., Zoller, M., Uhler, M. D., Helfman, D. M., McKnight, G. S. and Krebs, E. G. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 5192-5196
9. Øyen, O., Myklebust, F., Scott, J. D., Hansson, V. and Jahnsen, T. (1988) FEBS Lett. 246 (1,2) 57-64
10. Jahnsen, T., Hedin, L., Kidd, V., Beattie W.G., Lohmann, S.M., Walter, U., Durica, U., Schulz, T.Z., Schiltz, E., Browner, M., Lawrence, C.B., Goldman, D., Ratoosh, S.L. and Richards, J.S. (1986) J. Biol. Chem. 261, 12352-12361
11. Levy, F. O., Øyen, O., Sandberg, M., Taskén, K., Hansson, V. and Jahnsen, T. (1988) Mol. Endo., 2 (12), 1364-1373
12. Uhler, M. D., Carmichael, D. F., Lee, D. C., Chrivia, J. C., Krebs, E. G. and McKnight, G. S. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 1300-1304
13. Adavani, S. R., Schwarz, M., Showers, M. O., Maurer, R. A. and Hemmings, B. A. (1987) Eur. J. Biochem. 167, 221-226
14. Uhler, M. D., Chrivia, J. C. and McKnight, G. S. (1986) J. Biol. Chem. 261, 15360-15363
15. Showers, M. O. and Maurer, R. A. (1986) J. Biol. Chem. 261, 16288-16291
16. Beebe, S., Øyen, O., Sandberg, M., Frøysa, A., Hansson, V. and Jahnsen, T. (in press) Mol. Endo.
17. Øyen, O., Frøysa, A., Sandberg, M., Eskild, W., Joseph, D., Hansson, V. and Jahnsen, T. (1987) Biol. Reprod. 37, 947-956
18. Øyen, O., Scott, J., Cadd, G. G., McKnight, G. S., Krebs, E. G., Hansson, V. and Jahnsen, T. (1988) FEBS Lett. 229 (2), 391-394
19. Maniatis, T., Fritsch, E. F. and Sambrook, J. (1982) In Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
20. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 5463-5467

21. Tabor, S. and Richardson, C.C. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 4767-4771
22. Devereux, J., Haeberli, P. and Smithies, O. (1984) Nucleic Acids Res. 12, 387-395
23. Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutters, W.J. (1979) Biochemistry 18, 5294-5299
24. Breibart, R.E., Andreadis, A. and Nadal-Ginard, B. (1987) Ann. Rev. Biochem. 56, 467-495
25. Leff, S.E. and Rosenfeld, M.G. (1986) Ann. Rev. Biochem. 55, 1091-1117
26. Padgett, R.A., Grabowski, P.J., Konarska, M.M., Seiler, S. and Sharp, P.A. (1986) Ann. Rev. Biochem. 55, 1119-1150
27. Ross, J. (1988) Mol. Biol. Med., 1-14
28. Shaw, G. and Kamen, R. (1986) Cell 46, 659-667
29. Sinclair, G. D. and Dixon, G. H. (1982) Biochemistry 21, 1869-1877
30. Hecht, N. B. (1986) in: Molecular and cellular endocrinology of the testis (M. Stefanini et al., eds.), 199-213, Elsevier Science Publishers B. V., Amsterdam
31. Heidaran, M. A. and Kistler, W. S. (1987) J. Biol. Chem. 262, 13309-13315