responsible for the changing profile of pituitary GnRH receptors and responsiveness during physiological variations in reproductive function.

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References and Notes

1. R. N. Clayton, R. A. Shakespear, J. A. Duncan, J. C. Marshall, with an appendix by P. J. Munson and D. Rodbard, *Endocrinology* **105**, 1369 (1979).
2. Z. Naor, R. N. Clayton, K. J. Catt, *ibid.* **107**, 1144 (1980).
3. R. N. Clayton, A. R. Solano, A. Garcia-Vela, M. L. Dufau, K. J. Catt, *ibid.*, p. 699.
4. R. T. Savoy-Moore, N. B. Schwartz, J. A. Duncan, J. C. Marshall, *Science* **209**, 942 (1980).
5. J. Marian, R. L. Cooper, P. M. Conn, *Mol. Pharmacol.* **19**, 319 (1981).
6. R. N. Clayton and K. J. Catt, *Endocr. Rev.* **2**, 2 (1981).
7. ———, *Endocrinology* **108**, 887 (1981).
8. M. S. Frager, D. R. Pieper, S. A. Tonetta, J. A. Duncan, J. C. Marshall, *J. Clin. Invest.* **67**, 615 (1981).
9. T. M. Nett, M. E. Crowder, G. E. Moss, T. M. Duello, *Bio. Reprod.* **24**, 1145 (1981).
10. H. Nakane, C. P. Fawcett, S. M. McCann, *Endocrinology* **98**, 278 (1976).
11. The GnRH and analogs were as follows: the [D-Ser(t-Bu)^6]des-Gly^{10}-GnRH *N*-ethylamide (D-Ser^6-EA) was donated by J. Sandow, Hoechst A.G., Frankfurt/Main, West Germany; native GnRH, the agonist analog [D-Ala^6]des-Gly^{10}-GnRH *N*-ethylamide (D-Ala^6-EA), and the antagonist analog [D-Phe^2,Pro^3,D-Phe^6]GnRH were obtained from Peninsula Laboratories Inc., San Carlos, Calif.
12. Z. Naor, G. Childs, A. Leifer, R. N. Clayton, A. Amsterdam, K. J. Catt, *Mol. Cell. Endocrinol.*, in press.
13. E. Loumaye, Z. Naor, K. J. Catt, in preparation.
14. D. K. Sarkar, S. A. Chiappa, G. Fink, N. M. Sherwood, *Nature (London)* **264**, 461 (1976).
15. N. M. Sherwood and G. Fink, *Endocrinology* **106**, 363 (1980).
16. C. Mukherjee and R. Lefkowitz, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 1494 (1976).
17. T. M. Duello and T. M. Nett, *Mol. Cell. Endocrinol.* **19**, 101 (1980).
18. V. Giguere, F-A. Lefebvre, F. Labrie, *Endocrinology* **108**, 350 (1981).
19. T. E. Adams, R. L. Norman, H. G. Spies, *Science* **213**, 1388 (1981).
20. E.L. was the recipient of a Belgian-American Educational Foundation fellowship.

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αA-Crystallin Messenger RNA of the Mouse Lens: More Noncoding Than Coding Sequences

Abstract. *The 14S messenger RNA (1300 to 1500 nucleotides) for the αA chain of α-crystallin of the mammalian lens is nearly three times larger than required to code for the polypeptide that contains 173 amino acids. As a means of accounting for this anomaly, a complementary DNA clone for the mouse αA-crystallin messenger RNA was constructed in pBR322 and sequenced. Derivation of the protein sequence from the nucleic acid sequence showed that mouse αA-crystallin is similar to that of other organisms. The messenger RNA contains 536 nucleotides located on the 3′ side of the coding region, excluding the polyadenylate stretch. This 3′ sequence does not encode any other crystallin and has multiple termination codons in the three possible reading frames.*

The ocular lens of vertebrates synthesizes and accumulates large amounts of structural proteins called crystallins (*1*). The crystallins contribute 90 percent of the soluble lens protein and are highly conserved during evolution (*2*). There are four immunologically separate classes of crystallins called α-, β-, γ- and δ-crystallin. δ-Crystallin occurs only in birds and reptiles (*3*). The messenger RNA's (mRNA) for the δ-crystallins (*4*), the αB chain of α-crystallin, the β-crystallins, and the γ-crystallins (*5, 6*) are, like those of most eukaryotic mRNA's, only slightly larger than their coding requirements. By contrast, the mRNA for the αA chain of α-crystallin from calves (*7*) and rats (*8*) has a sedimentation value of 14*S* (1300 to 1500 nucleotides), which is nearly three times the size necessary to code for the 173 amino acids of this polypeptide (*9*). The 14*S* αA-crystallin mRNA is capped (*10*) and polyadenylated (*11*). The evolutionary conservation of the large size of the αA-crystallin mRNA suggests a critical function outside of the region coding for αA-crystallin. It has been suggested that the 14*S* mRNA may be bicistronic and encode two α-type crystallin polypeptides (*12*). To gain insight into the function of the sequences of the 14*S* αA-crystallin mRNA, we have constructed and sequenced a complementary DNA (cDNA) clone for this mRNA from the mouse lens.

Polyadenylated mRNA from 1000 lenses of 5- to 10-day-old mice (NIH general-purpose stock) was extracted, subjected to reverse transcription, made double-stranded with DNA polymerase I of *Escherichia coli*, and inserted into the Pst I site of the plasmid pBR322 by the G-C (guanine-cytosine) procedure (*13*). The recombinant plasmids were used to transform *E. coli* LE392. More than 1000 recombinant cDNA clones were obtained. A putative αA-crystallin cDNA clone was identified by the technique of positive hybrid-selection (*14*), with a rabbit reticulocyte lysate (New England Nuclear) as the cell-free protein synthesizing system. A recombinant plasmid (pMαACr2) selectively hybridized mRNA from the mouse lens that directed the synthesis of αA crystallin in vitro (data not shown). Nucleotide sequence of the pMαACr2 insert was determined by the method of Maxam and Gilbert (*15*) (Fig. 1). The sequence and translation of the cloned cDNA in pMαACr2 are shown in Fig. 2a. Comparison with the amino acid sequence for the αA chain of α-crystallin of other mammals (*16*) revealed that pMαACr2 contains nearly a complete copy of the codons for this polypeptide. Nucleotides 1 to 490 (Fig. 2a) predict an amino acid sequence identical to amino acids 10 to 173 of rat αA-crystallin (*16*). This result is consistent with the finding that the evolution of α-crystallin is very slow (*2*). It has been estimated from protein sequencing studies that amino acid substitutions in α-crystallin occur at a rate of approximately 1 percent per 17 million years (*2*). Since rats and mice are believed to have diverged about 10 million years ago (*17*), we would not expect more than a single amino acid difference in the αA-crystallin polypeptides of these rodents. The nucleotide sequence of pMαACr2 also reveals an unusually long sequence 3′ to the coding region. The polyadenylation signal AATAAA (A, adenine; T, thymine), which is present in all eukaryotic mRNA's (*18*), is present in the 3′ region starting at position 1002 in Fig. 2a.

We examined all three possible reading frames of the cloned insert in pMαACr2 for initiation and termination codons to test the possibility that another protein may be encoded within this cDNA. The results demonstrated that multiple termination signals exist in the nucleotide sequence 3′ to the αA-crystallin coding region, regardless of the reading frame considered (Fig. 2b). Only reading frame 1 lacks a termination codon within the known coding region. Considering the location of possible initiation and termination codons on the 3′ side of the coding region, the largest polypeptide that could be synthesized with any reading frame is 60 amino acids (reading frame 3 in Fig. 2b).

A possible criticism of this stop codon analysis is its sensitivity to single nucleotide errors in the sequence determination. An added or deleted base shifts the termination codon into a new reading frame. To investigate the possibility that nucleotide sequences coding for crystallin polypeptides are contained in the cloned cDNA in pMαACr2 we used a dot matrix computer analysis that de-

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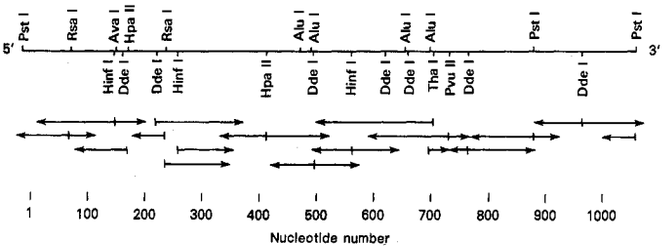
Fig. 1. Restriction map and strategy used to determine the nucleotide sequence of pMαACr2. DNA fragments were isolated from restriction enzyme digestions of pMαACr2 by electrophoresis on a polyacrylamide gel solubilized with β-mercaptoethanol (*28*). Restriction enzymes were purchased from New England Biolabs and Bethesda Research Laboratories. The purified fragments were radioactively labeled by means of T_4 polynucleotide kinase (P-L Biochemicals) and [γ-^{32}P]adenosine triphosphate (Amersham/Searle). Each arrow represents the direction of sequencing of the isolated fragments. The sequence was determined by autoradiographic detection of the chemical cleavage products separated on 20 percent and 10 percent polyacrylamide gels containing 8*M* urea (*15*).



tects homologies between related amino acid sequences (*19*). The three potential reading frames of the pMαACr2 sequence were searched for homologies to the known protein sequences of bovine αA- (*9*), αB- (*20*), βBp- (*21*), and γ-crystallins (*22*). As would be expected, the amino acid sequence encoded in the αA-crystallin coding block of pMαACr2 showed extensive homology to the αA chain and partial homology to the αB chain of bovine α-crystallin (data not shown); no homology with α-crystallin was found in the 3′ flanking sequences. When all possible amino acid sequences were derived from the nucleotide sequence of the cDNA in pMαACr2, no obvious homologies could be detected with the amino acid sequences of calf β- or γ-crystallins.

We have determined the size of the mouse αA-crystallin mRNA by denaturing gel electrophoresis (*23*) and blot hybridization (*24*) in order to estimate the number of nucleotides missing from pMαACr2. The αA-crystallin mRNA was approximately 1420 nucleotides long (data not shown). Thus, pMαACr2 lacks about 370 nucleotides of the αA-crystallin mRNA. Since the calf 14*S* αA-crystallin mRNA has an average polyadenylate length of 200 nucleotides (*11*), we estimate that there are 100 to 200 nucleotides on the 5′ side of the coding region in the mouse αA-crystallin mRNA.

Our results indicate that the 14*S* mouse αA-crystallin mRNA does not encode more than one crystallin polypeptide even though there are almost three times more nucleotides than required to synthesize αA-crystallin. Most of the additional length of the mRNA

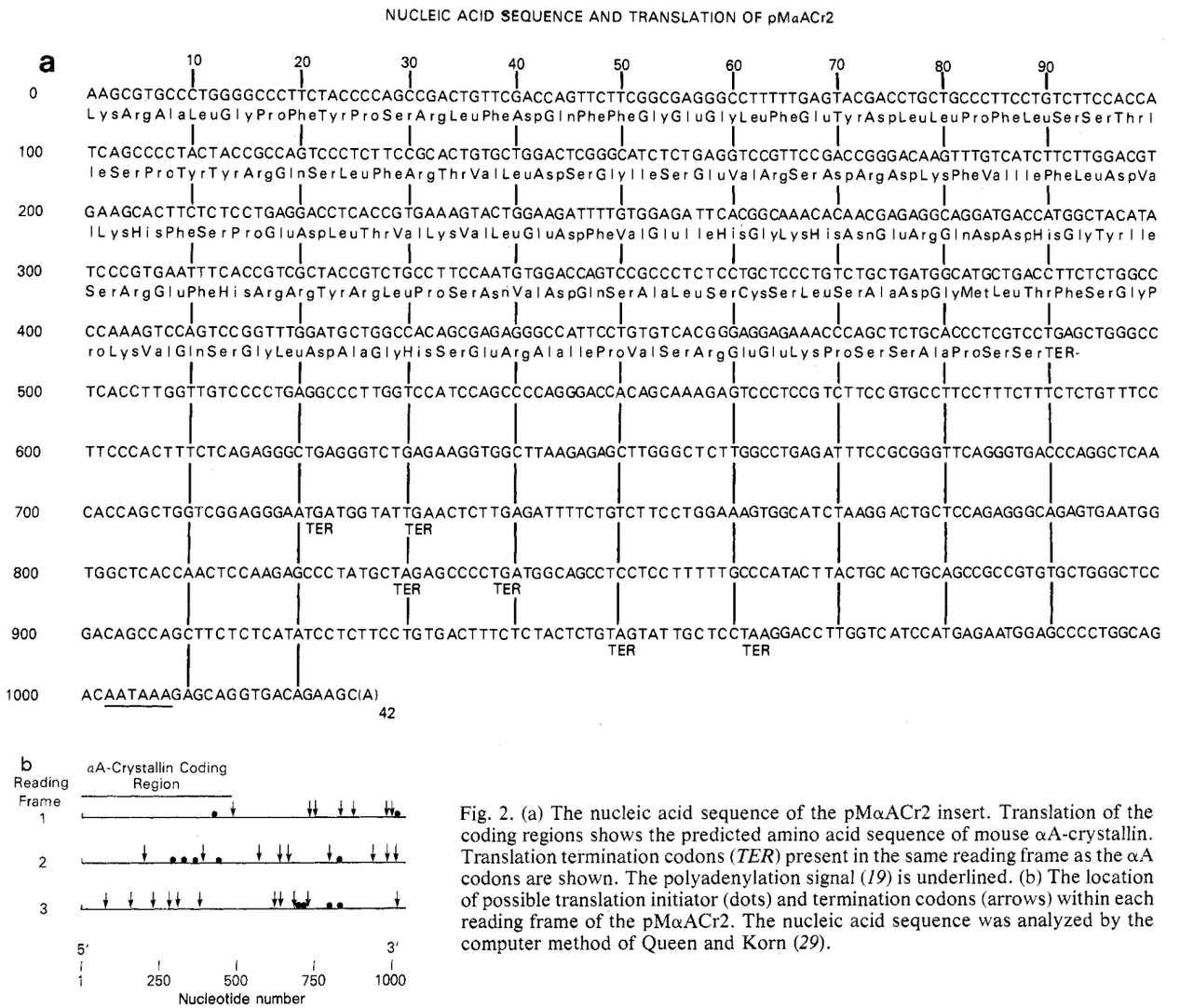


Fig. 2. (a) The nucleic acid sequence of the pMαACr2 insert. Translation of the coding regions shows the predicted amino acid sequence of mouse αA-crystallin. Translation termination codons (*TER*) present in the same reading frame as the αA codons are shown. The polyadenylation signal (*19*) is underlined. (b) The location of possible translation initiator (dots) and termination codons (arrows) within each reading frame of the pMαACr2. The nucleic acid sequence was analyzed by the computer method of Queen and Korn (*29*).

resides in the 536 nucleotides and the polyadenylate stretch that are present on the 3′ side of the coding region. At present we cannot rule out the possibility that a small noncrystallin polypeptide is coded within this region. Although the length of this 3′ region is large in comparison with numerous other mRNA's, such as the 92-nucleotide untranslated region of rabbit β-globin mRNA (*25*), it is not unprecedented among eukaryotic mRNA's. For example, the 3′ untranslated sequence of chicken ovalbumin mRNA consists of 634 nucleotides (*26*). Also, the length of the 3′ untranslated region for the dihydrofolate reductase mRNA in the mouse ranges from about 80 nucleotides to about 930 nucleotides in the four different RNA's found for this enzyme (*27*). The size of the polypeptide encoded by the dihydrofolate reductase mRNA's is approximately the same as αA-crystallin. The function of the sequences on the 3′ side of the coding region is not known yet for any mRNA. Possibly, comparisons of the 3′ sequences of the 14*S* mRNA for α-crystallin from different organisms will reveal stretches of conserved nucleotides and provide insight as to their functional significance.

Note added in proof: After submission of this report, the sequence of a rat αA-crystallin cDNA was reported (*30*). The findings were similar to those reported here. Comparison of the 3′ noncoding regions of the mouse and rat αA-crystallin cDNA's show that 24 insertion-deletion and 32 base change events have occurred during the evolutionary divergence of these organisms. There are 18 synonymous base differences within the encoding region.

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References and Notes

1. J. J. Harding and K. J. Dilley, *Exp. Eye Res.* **22**, 1 (1976); H. Bloemendal, *Science* **197**, 127 (1977).
2. W. W. de Jong, in *Molecular and Cellular Biology of the Eye Lens*, H. Bloemendal, Ed. (Wiley, New York, 1981), p. 221.
3. W. Manski and S. P. Halbert, *Protides Biol. Fluids Proc. Colloq.* **12**, 117 (1964); R. M. Clayton, in *The Eye*, H. Davson and L. T. Graham, Eds. (Academic Press, New York, 1974), p. 399; L. A. Williams and J. Piatigorsky, *Eur. J. Biochem.* **100**, 349 (1979).
4. P. Zelenka and J. Piatigorsky, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1896 (1974); R. Reszelbach, T. Shinohara, J. Piatigorsky, *Exp. Eye Res.* **25**, 583 (1977).
5. H. Bloemendal, in *Molecular and Cellular Biology of the Eye Lens*, H. Bloemendal, Ed. (Wiley, New York, 1981) p. 189.
6. H. J. Dodemont, P. M. Andreoli, R. J. M. Moormann, F. C. S. Ramaekers, J. G. G. Schoenmakers, H. Bloemendal, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5320 (1981).
7. A. J. M. Berns, G. J. A. M. Strous, H. Bloemendal, *Nature New Biol.* **236**, 7 (1972); A. Berns, P. Janssen, H. Bloemendal, *Biochem. Biophys. Res. Commun.* **59**, 1157 (1974); J. H. Chen and A. Spector, *Biochemistry* **16**, 499 (1977).
8. L. H. Cohen, D. P. E. M. Smits, H. Bloemendal, *Eur. J. Biochem.* **67**, 563 (1976).
9. F. J. van der Ouderaa, W. W. de Jong, H. Bloemendal, *ibid.* **39**, 207 (1973).
10. F. A. M. Asselbergs *et al.*, *Biochim. Biophys. Acta* **520**, 577 (1978).
11. A. Favre, U. Bertazzoni, A. J. M. Berns, H. Bloemendal, *Biochem. Biophy. Res. Commun.* **56**, 273 (1974).
12. J. H. Chen and A. Spector, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5448 (1977).
13. S. P. Bhat and J. Piatigorsky, *ibid.* **76**, 3299 (1979); H. W. Boyer *et al.*, in *Recombinant Molecules: Impact on Science and Society*, R. F. Beers and E. G. Basset, Eds. (Raven, New York, 1977), p. 9.
14. J. Parnes *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2253 (1981).
15. A. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980).
16. W. W. de Jong, F. J. van der Ouderaa, M. Versteeg, G. Groenewoud, J. M. van Amelsvoort, H. Bloemendal, *Eur. J. Biochem.* **53**, 237 (1975).
17. D. E. Kohne, *Q. Rev. Biophy.* **3**, 327 (1970); E. Brownell, thesis, Yale University (1980).
18. N. J. Proudfoot and G. G. Brownlee, *Nature (London)* **263**, 211 (1976).
19. J. V. Maizel, Jr., and R. Lenk, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
20. F. J. van der Ouderaa, W. W. de Jong, A. Hilderink, H. Bloemendal, *Eur. J. Biochem.* **49**, 157 (1974).
21. H. P. C. Driessen, P. Herbrink, H. Bloemendal, W. W. de Jong, *Exp. Eye Res.* **31**, 243 (1980).
22. L. R. Croft, *Biochem. J.* **128**, 961 (1972).
23. H. Lehrach, D. Diamond, J. N. Wozney, H. Boedtker, *Biochemistry* **16**, 4743 (1977).
24. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
25. N. J. Proudfoot, *Cell* **10**, 559 (1977).
26. L. McReynolds *et al.*, *Nature (London)* **273**, 723 (1978).
27. D. R. Setzer, M. McGrogan, J. H. Numberg, R. T. Schimke, *Cell* **22**, 361 (1980).
28. J. N. Hansen, B. H. Pheiffer, J. A. Boehnert, *Anal. Biochem.* **105**, 192 (1980).
29. C. L. Queen and L. J. Korn, *Methods Enzymol.* **65**, 595 (1980).
30. R. J. M. Moormann *et al.*, *Nucleic Acids Res.* **9**, 4813 (1981).
31. We thank Dr. J. V. Maizel, Jr., and B. Norman for help with the dot matrix, Drs. P. Hieter and R. E. Jones for technical advice, and Dr. C. G. Sibley for a discussion concerning the divergence of rats and mice.

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Cardiac Catabolic Factors: The Degradation of Heart Valve Intercellular Matrix

Abstract. *Cultures of porcine heart valves and aorta secrete a factor that stimulates the degradation of cartilage matrix in a fashion similar to that displayed by synovial catabolin. The heart valve factor also induces the release of chondroitin sulfate and hydroxyproline from isolated heart valve cultures. The present observations support the hypothesis that tissues producing catabolic factors (catabolins) may well be responsive to them and that these messengers may play a role in the cellular regulation of the degradation of intercellular macromolecules.*

Our present understanding of the mechanisms associated with the degradation of intercellular macromolecules stems largely from observations on cartilage matrix breakdown. Until recently the local invasion of the articular cartilage by synovial pannus was believed to be responsible for the degradation of proteoglycan and collagen that constitute the major structural components of the matrix. However, Fell and Jubb (*1*) and somewhat later Steinberg's group (*2*) demonstrated that normal as well as rheumatoid synovium secreted a diffusible product which induced chondrocytes to destroy their own matrix. Dingle and co-workers (*3–5*) have isolated and partially characterized a small acidic protein (catabolin) from porcine synovium which also stimulates viable chondrocytes to rapidly degrade their matrix. Although it is apparent that synovial catabolin initiates the breakdown of cartilage matrix in vitro, it is not known whether there is a more fundamental role for such factors in regulating the content and distribution of intercellular macromolecules in other tissues. It is possible that autologous and heterologous classes of catabolin-like factors mediate the intercellular turnover of proteoglycan and collagen, and that the interaction of "catabolins" with other factors that promote the synthesis of matrix components control the composition of connective tissue elements under altering physiolog-

Table 1. Tissues and cells producing and responding to catabolin. N.D., not determined.

Tissues or cells	Production	Response
Synovium (human)	+	N.D.
Synovium (pig)	+	+
Synovium (rabbit)	+	N.D.
Cartilage (human)	N.D.	+
Cartilage (pig)	N.D.	+
Cartilage (rabbit)	N.D.	+
Heart valve (pig)	+	+
Blood vessels (pig)	+	+
Placenta	+	N.D.
Kidney	+	N.D.
Fibroblasts (human synovial)	+	N.D.
Fibroblasts (pig synovial)	+	N.D.
Blood vessel (smooth muscle cells)	+	N.D.
Blood vessel (endothelium cells)	+	N.D.
Activated monocytes	+	+

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