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Structural and Functional Similarities of δ-Crystallin Messenger Ribonucleic Acids from Duck and Chicken Lenses†

Joram Piatigorsky

ABSTRACT: δ-Crystallin of the embryonic duck lens was compared with that of the embryonic chicken lens with respect to polypeptide composition, synthesis, and messenger ribonucleic acid (mRNA) sequences. Labeling experiments with [^{35}S]-methionine revealed that the duck δ-crystallin is composed of minor amounts of polypeptides with molecular weights near 50 000 (50K) and 49 000 (49K) and much greater amounts of polypeptides with molecular weights near 48 000 (48K) and 47 000 (47K), as judged by sodium dodecyl sulfate–urea–polyacrylamide gel electrophoresis. All four sizes of polypeptides were synthesized in similar relative proportions as found in vivo in a rabbit reticulocyte lysate supplemented with δ-crystallin mRNA isolated from the embryonic duck lens. Synthesis of the 48K and 47K δ-crystallin polypeptides was differentially reduced in duck lenses cultured in the presence of ouabain. This is similar to the differential reduction of synthesis of the lower molecular weight δ-crystallin peptides in embryonic chicken lenses demonstrated previously. R loops formed between duck or chicken δ-crystallin mRNA and a cloned chicken δ-crystallin cDNA and heteroduplexes formed between duck or chicken δ-crystallin mRNA and cloned chicken genomic DNAs containing δ-crystallin sequences showed that, except for the putative 5′ leader sequence, the duck and chicken δ-crystallin mRNAs have extremely similar nucleotide sequences. These data indicate considerable conservation of δ-crystallin throughout the approximately 100 million years of divergence between ducks and chickens. The findings also suggest a possible relationship between the structure of δ-crystallin mRNA and the differential reduction in synthesis of the lower molecular weight δ-crystallin polypeptides in ouabain-treated lenses of ducks and chickens.

Crystallins are highly conserved structural proteins which comprise at least 90% of the soluble protein of the vertebrate lens [see Harding & Dilley (1976) and Bloemendal (1977)]. There are four families of crystallins (α-, β-, γ-, and δ-crystallin) which differ in their biochemical and immunological properties. Each crystallin family is composed of multiple polypeptides.

δ-Crystallin differs from the other crystallins in that it is found only in the lenses of birds and reptiles [see Clayton (1974) and Williams & Piatigorsky (1979a)]. This crystallin has been most intensively studied in the chicken. Chicken δ-crystallin is a tetrameric protein with a native molecular weight near 200 000 (200K) (Piatigorsky et al., 1974). The δ-crystallin polypeptides may be fractioned into a minor band of 50K polypeptides and a major band of 48K polypeptides by sodium dodecyl sulfate ($NaDodSO_4$)–urea–polyacrylamide gel electrophoresis (Reszelbach et al., 1977). Both δ-crystallin polypeptides are synthesized in a heterologous cell-free system, suggesting that each is encoded by a separate mRNA (Reszelbach et al., 1977). The δ-crystallin polypeptides are extremely similar (Piatigorsky, 1976) and, to date, have been found to differ from each other by only two tryptic peptides (Shinohara et al., 1980). Despite their similarity, the ratio of synthesis of the δ-crystallin polypeptides is markedly affected by the relative concentrations of Na^+, Cl^-, K^+, and acetate in the cultured embryonic chicken lens (Shinohara & Piatigorsky, 1977; Shinohara et al., 1980) and in a reticulocyte lysate containing δ-crystallin mRNA (Shinohara & Piatigorsky, 1980). The basis for this unexpected finding is not known but may reflect some interesting structural feature(s) of the δ-crystallin mRNA(s).

In the present investigation, I have examined δ-crystallin synthesis in the duck in order to determine whether the ionically controlled alteration in the ratio of synthesis of the δ-crystallin polypeptides is confined to chickens or whether it is an evolutionarily conserved characteristic found in other species. Like chicken δ-crystallin, duck δ-crystallin is a tetramer with a molecular weight of approximately 200K and is fractionated by $NaDodSO_4$–urea–polyacrylamide gel electrophoresis into at least two bands with molecular weights near 47K and 48K (Wiliiams & Piatigorsky, 1979b). These duck

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δ-crystallin polypeptides are present in nearly equimolar amounts as judged by staining the polyacrylamide gel with Coomassie blue. Here it is shown by radioactive labeling experiments that duck δ-crystallin contains two additional minor bands with molecular weights near 49K and 50K. Interestingly, the 49K and 50K δ-crystallin polypeptides are synthesized preferentially in the embryonic lenses treated with ouabain, indicating that the ionic control of δ-crystallin synthesis occurs in duck as well as in chicken lenses. Hybridization of duck δ-crystallin mRNA to a cloned chicken δ-crystallin cDNA (pδCr2) (Bhat & Piatigorsky, 1979) and to cloned chicken genomic fragments containing δ-crystallin sequences (Bhat et al., 1980; Jones et al., 1980) showed, in addition, that the δ-crystallin mRNA sequences of the duck are very similar to those of the chicken.

Material and Methods

Obtaining and Labeling the Embryonic Duck Lenses. Peking duck embryos (*Anas platyrhynchos*) were obtained from Truslow Farms, Inc., Chestertown, MD. In vivo labeling experiments were performed with 7- and 14-day-old embryonic lenses. A total of 4–22 lenses were precultured for 6 h in tissue culture dishes (Falcon Plastics, 15 × 60 mm) containing 2.0 mL of Ham's F-10 medium (Ham, 1963) at 37 °C in a 5% CO_2–95% air atmosphere in the presence or absence of 10^{-4} M ouabain (Sigma Chemical Co., St. Louis, MO); labeling was for an additional 6 h with 500 μCi of [^{35}s]methionine (New England Nuclear Corp.; 500–600 Ci/mmol).

Polyacrylamide Gel Electrophoresis. For analysis of δ-crystallin, homogenates were centrifuged at 10000 rpm for 10 min, and the pH 5 soluble fraction [see Piatigorsky et al. (1974)] of the supernatant fraction was subjected to electrophoresis. Discontinuous electrophoresis was performed in 10% polyacrylamide gel slabs containing 0.1% $NaDodSO_4$ (Pierce Chemical Co.) and ultrapure 8 M urea (Schwarz/Mann) as described elsewhere (Reszelbach et al., 1977). Samples were dissolved in 1% $NaDodSO_4$, 4 M urea, 1% 2-mercaptoethanol, 10% glycerol, and 0.6% Tris-HCl, pH 6.8, and heated to 100 °C for 2–3 min before electrophoresis. After electrophoresis, the gels were soaked in 5% acetic acid and 5% methanol to remove the urea, stained in Coomassie brilliant blue R, dried under vacuum, and subjected to autoradiography with Kodak SB-5 X-ray film.

Preparation of mRNA. Cytoplasmic RNA was extracted with buffer-saturated (0.01 M Tris-HCl, pH 7.5, 0.1 M NaCl, and 1 mM EDTA) phenol–chloroform–isoamyl alcohol (50:50:1) from the 10000*g* supernatant fraction of 500 14-day-old embryonic duck lenses (Zelenka & Piatigorsky, 1974). The lenses were stored in liquid nitrogen before extraction. The polyadenylated RNA was purified from the cytoplasmic RNA by oligo(dT)–cellulose (P-L Biochemicals) chromatography (Zelenka & Piatigorsky, 1974), ethanol precipitated after adjustment to 1% potassium acetate, lyophilized, redissolved in sterile water, and kept in 6-μL aliquots in liquid nitrogen. Approximately 75 μg of polyadenylated RNA was obtained from the 500 embryonic duck lenses.

Cell-Free Translation of mRNA. Polyadenylated RNA was translated in a total volume of 50 μL for 1 h at 37 °C in a rabbit reticulocyte lysate as described by Zelenka & Piatigorsky (1974), except that 1 μg of rat liver tRNA was added per reaction mixture. Each reaction contained 25 μCi of [^{35}S]methionine (New England Nuclear, 500 Ci/mmol). Aliquots were examined directly by $NaDodSO_4$–urea–polyacrylamide gel electrophoresis or were first immunoprecipitated with anti-chicken δ-crystallin sheep antiserum as given below. Nonradioactive chicken δ-crystallin (2 μg) was added to the reaction mixture before immunoprecipitation.

Immunoprecipitation of δ-Crystallin. δ-Crystallin antiserum was made in sheep against purified embryonic chicken δ-crystallin (Milstone & Piatigorsky, 1975). The chicken δ-crystallin was purified by isoelectric focusing of the native protein (200000 daltons) in a sucrose density gradient (Piatigorsky et al., 1974). The anti-chicken δ-crystallin antiserum cross-reacts with duck δ-crystallin (Williams & Piatigorsky, 1979b). Nonradioactive chicken δ-crystallin (5 μg) was added to the supernatant fractions before immunoprecipitation. The immunoprecipitation reaction mixtures contained 0.1 M Tris-HCl, pH 7.5, 0.05 M NaCl, 0.025 M L-methionine, 0.1 M Na_4EDTA, 1% Triton X-100, 0.5% deoxycholate, and 0.25% bovine serum albumin. The pellets were centrifuged through 1 M sucrose.

Electron Microscopy. R loops were formed with duck δ-crystallin mRNA and pδCr2 as described by Bhat & Piatigorsky (1979). pδCr2 was linearized with *Bam*HI endonuclease; 1 μg of pδCr2 was then hybridized to 1 μg of mRNA in a total of 20 μL containing 70% formamide (deionized), 0.1 M *N*-tris(hydroxymethyl)methylglycine (Tricine)–NaOH at pH 8.0, 0.5 M NaCl, and 10 mM EDTA. Hybridization was for 12–14 h at 52 °C in a sealed capillary tube.

RNA–DNA heteroduplexes were formed with duck δ-crystallin mRNA and chicken genomic clones as given by Bhat et al. (1980). The hybridization reactions were conducted with 1 μg of the cloned genomic fragments, 1 μg of Ch 4A DNA, and 1–2 μg of duck δ-crystallin mRNA in 70% formamide (deionized), 0.1 M Tris-HCl at pH 7.5, 0.15 M NaCl, and 0.01 M EDTA in a total of 100 μL. Hydridization was for 15 min at 85 °C followed by 2 h at 43 °C. The chicken δ-crystallin clones gδCr1 and gδCr2 have been described by Bhat et al. (1980), and the chicken library *Hae*III clone has been described by Jones et al. (1980). The molecules were measured with a Numonics digitizer.

Results

δ-Crystallin Synthesis in the Cultured Embryonic Duck Lens. An earlier study (Williams & Piatigorsky, 1979b) has shown that δ-crystallin in the embryonic duck lens can be fractionated into two bands (47K and 48K) with equal staining intensities by $NaDodSO_4$–urea–polyacrylamide gel electrophoresis (Figure 1A). The autoradiogram in Figure 1B shows, as expected, that equivalent amounts of the 47K and 48K bands of δ-crystallin are synthesized in the cultured embryonic duck lens. In addition, close inspection of the autoradiogram reveals two trace bands of labeled protein just above the 47K and 48K bands of δ-crystallin. In separate tests, these minor bands of ^{35}S-labeled protein immunoprecipitated with anti-chicken δ-crystallin antiserum (not shown). These data indicate that embryonic duck δ-crystallin is composed of polypeptides that can be resolved into at least four sizes ranging from approximately 47K to 50K in molecular weight.

In order to test whether the ratio of synthesis of the δ-crystallin polypeptides is affected by the intracellular concentrations of electrolytes in ducks, as it is in chickens (Shinohara & Piatigorsky, 1977), the embryonic duck lens was treated with 10^{-4} M ouabain for 6 h and then labeled with [^{35}S]methionine for 6 h. Ouabain inhibits (Na^+,K^+)-ATPase and results in the equilibration of the intracellular and extracellular Na^+ and K^+ concentrations. In the medium used in the present study (Ham's F-10), ouabain treatment causes the intracellular concentration of Na^+ to approach 150 mM and that of K^+ to decrease to approximately 3 mM in the cultured lenses (Shinohara & Piatigorsky, 1977). Ouabain treatment inhibited incorporation of [^{35}S]methionine into the

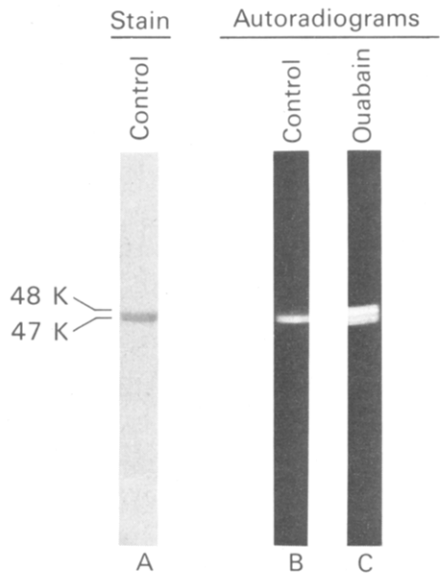

FIGURE 1: $NaDodSO_4$–urea–polyacrylamide gel electrophesis of δ-crystallin from 7-day-old embryonic duck lenses. A total of 22 lenses were incubated at 37 °C in Ham's F-10 for 6 h and subsequently labeled with 500 μCi of [^{35}S]methionine (581 Ci/mmol) for 6 h in the presence or absence of 10^{-4} M ouabain. After electrophoresis, the gels were either stained or subjected to autoradiography, as indicated. Chicken δ-crystallin was analyzed in a parallel slot in order to provide 50K and 48K markers. (B) Exposed for 1 day; (C) exposed for 10 days. The staining pattern was the same in the gels containing protein of the control or ouabain-treated lenses.

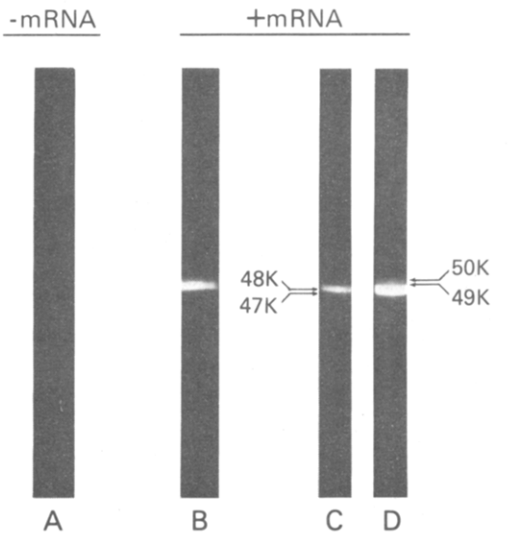

FIGURE 2: Autoradiograms of $NaDodSO_4$–urea–polyacrylamide gels of translation products of polyadenylated RNA from 14-day-old embryonic duck lenses. The proteins were synthesized in a rabbit reticulocyte lysate and immunoprecipitated with anti-chicken δ-crystallin sheep antiserum as given under Materials and Methods. (A, B, C) Exposed for 1 day; (D) exposed for 4 days. Arrows on (C) denote the two principal bands of δ-crystallin; arrows on (D) denote the two, faint higher molecular weight bands of δ-crystallin. The higher molecular weight bands were clearly discernible in the original autoradiograph. (B) Total translation; (C, D) immunoprecipitate.

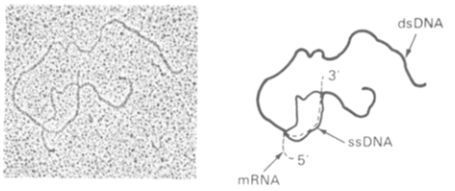

FIGURE 3: R loop formed by hybridization of duck δ-crystallin mRNA with a cloned chicken δ-crystallin cDNA (pδCr2). Orientation of the 5′ and 3′ ends of the inserted cDNA is from Bhat & Piatigorsky (1979). The 3′-RNA tail presumably contains the poly(A) sequences.

total protein of the cultured duck lens by at least 90%. Interestingly, in the ouabain-treated lens, the relative amount of [^{35}S]methionine incorporated into the two higher molecular weight δ-crystallin polypeptides was greatly increased and actually exceeded that incorporated into the 47K and 48K polypeptides (Figure 1C). A faint band of about 52K was also evident in the autoradiogram of the proteins from the ouabain-treated lenses. All these polypeptides labeled in the presence of ouabain precipitated with anti-δ-–crystallin antiserum (not shown). Thus, the ionic control of the ratio of synthesis of the δ-crystallin polypeptides is not limited to chickens.

Cell-Free Translation of δ-Crystallin mRNA. Polyadenylated RNA was extracted from 14-day-old embryonic duck lenses and translated in a rabbit reticulocyte lysate containing [^{35}S]methionine. After 1 h, 1 μg of nonradioactive δ-crystallin was added as carrier. The in vitro products either were analyzed directly by $NaDodSO_4$–urea–polyacrylamide gel electrophoresis (Figure 2B) or were precipitated with anti-chicken δ-crystallin antiserum, and the immunoprecipitate was subjected to $NaDodSO_4$–urea–polyacrylamide gel electrophoresis (Figure 2C,D). The 47K and 48K polypeptides were the principal proteins synthesized in vitro and were made in approximately equal amounts (Figure 2B). Resolution of the two bands is best seen in the autoradiogram of the δ-crystallin immunoprecipitate that was developed for 1 day (Figure 2C). Longer exposures of autoradiograms of the total in vitro products (not immunoprecipitated) revealed additional bands of lower molecular weight polypeptides corresponding to the β-crystallins (not shown). Close inspection of the autoradiograms indicated that trace amounts of label were detectable immediately above the 47K and 48K polypeptides. Exposure of the autoradiograms of the δ-crystallin immunoprecipitate for 4 days made it possible to distinguish discrete 49K and 50K δ-crystallin polypeptides in the gel (Figure 2D). Synthesis of these four polypeptides was dependent upon addition of the duck mRNA to the reticulocyte lysate (Figure 2A). These data suggest that each of the δ-crystallin polypeptides is synthesized by a different mRNA.

Hybridization of Duck δ-Crystallin mRNA to Cloned Chicken δ-Crystallin DNA Sequences. In order to test the extent of structural homology between the δ-crystallin mRNAs of the duck and the chicken, duck δ-crystallin mRNA was hybridized to cloned δ-crystallin DNA sequences of the chicken and examined in the electron microscope. First, duck δ-crystallin mRNA was hybridized to a cloned chicken δ-crystallin cDNA (pδCr2) under conditions allowing the formation of R loops. The duck mRNA–chicken cDNA hybrids in the heterologous R loops (Figure 3) measured 866 ± 29 (±SEM) base pairs (average of 28 molecules), revealing extensive homology between the δ-crystallin mRNA sequences of the duck with those of the chicken. Previous homologous R loops generated by hybridization of chicken δ-crystallin mRNA with pδCr2 measured 1241 ± 240 base pairs (average of 69 molecules) (Bhat & Piatigorsky, 1979). If one considers the error in the measurements (SEM), these data indicate that the maximum homology between the cloned region (~3′ half) of the δ-crystallin cDNA of the chicken and a similar region of δ-crystallin mRNA of the duck is 88%. This ignores the mismatching of bases thay may occur within the hybridized region of the R loops.

Next, duck δ-crystallin mRNA was hybridized to cloned chicken genomic *Eco*RI fragments containing δ-crystallin sequences (gδCr1, gδCr2) to test whether the homology be-

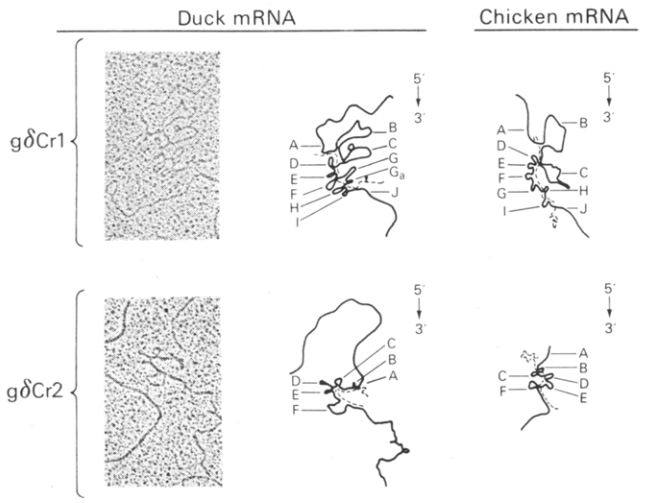

FIGURE 4: Heteroduplexes formed with duck δ-crystallin mRNA and cloned chicken genomic DNA sequences containing δ-crystallin sequences. Tracings of heteroduplexes using chicken δ-crystallin mRNA are shown for comparison [taken from Bhat et al. (1980)]. gδCr1 contains δ-crystallin gene sequences that are 5′ to those in gδCr2. gδCr1 lacks the putative leader sequence of this δ-crystallin gene.

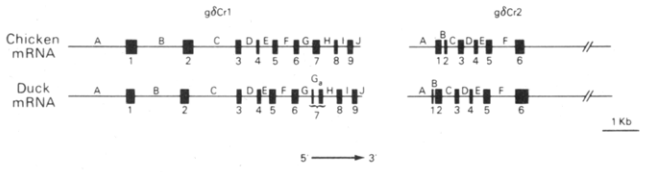

FIGURE 5: Diagrammatic representation of intervening (—) and mRNA gene (■) sequences in cloned chicken δ-crystallin DNAs hybridized with δ-crystallin mRNA from chickens or ducks. The diagrams were constructed from measurements made with a Numonics digitizer from electron micrographs as shown in Figure 4. Measurements of the heteroduplexes using duck mRNA are averages of 27 molecules using gδCr1 and 23 molecules using gδCr2. The measurements of heteroduplexes using chicken mRNA are from Bhat et al. (1980).

tween the δ-crystallin sequences of the chicken and the duck was sufficient to generate the same pattern of intervening sequences as observed previously (Bhat et al., 1980; Jones et al., 1980) with the homologous hybrids. Figure 4 shows electron micrographs of duck δ-crystallin mRNA hybridized to gδCr1 and gδCr2. gδCr1 contains two-thirds of the 5′ portion of a δ-crystallin gene, although it lacks at least one 5′ mRNA exon (presumably the leader sequence); gδCr2 contains the 3′ third of a different δ-crystallin gene (Jones et al., 1980). The exons of the two δ-crystallin genes from which these DNA fragments were derived cross-hybridize (Jones et al., 1980). The patterns of intervening sequences generated in the cloned chicken genomic fragments by the duck δ-crystallin mRNA were extremely similar to those obtained with the chicken δ-crystallin mRNA. For comparison, both the heterologous and the homologous hybrids are traced in Figure 4 and diagrammed in Figure 5. Virtually every loop of intervening sequence observed in the hybrids with the chicken mRNA was present in the hybrids with the duck mRNA. A few small deviations were observed in the lengths of the mRNA gene sequences and the intervening sequences between the heterologous and the homologous hybrids. These may represent errors in the measurements. Also, exon 7 in gδCr1 had a region of abut 100 nucleotides that did not hybridize to the duck mRNA.

Duck δ-crystallin mRNA was also hybridized to a cloned *Hae*III-derived fragment of chicken DNA containing the sequences in gδCr2 and the putative 5′ leader sequences of the

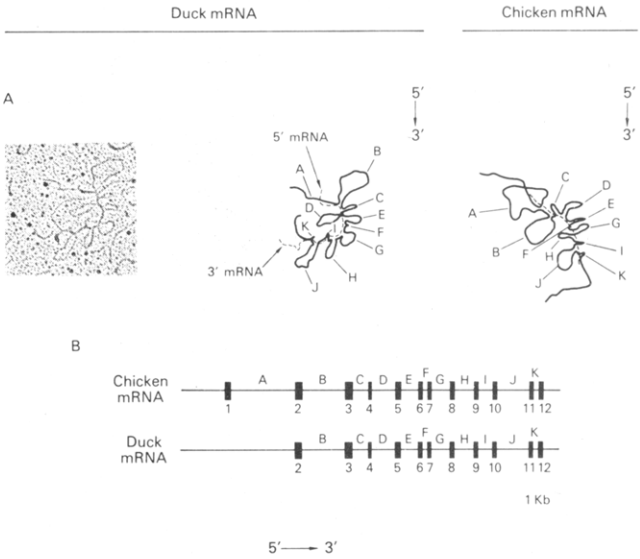

FIGURE 6: (A) Heteroduplexes formed with duck δ-crystallin mRNA and a cloned *Hae*III DNA fragment containing δ-crystallin sequences. A tracing of a heteroduplex using chicken δ-crystallin mRNA is shown for comparison [taken from Jones et al. (1980)]. (B) Diagrammatic representation of intervening (—) and mRNA gene (■) sequences in the heteroduplexes shown in (A). The values for the heteroduplex formed with duck mRNA are taken from measurements made from 16 molecules. The diagram for the heteroduplex with chicken mRNA is from Jones et al. (1980).

gene (Jones et al., 1980). As shown in Figure 6, the heterologous hybrid appeared the same as the homologous hybrid, with one exception. The extreme 5′ loop representing intervening sequence A generated by hybridization of the chicken δ-crystallin mRNA was not present when the duck δ-crystallin mRNA was used. This implies that the 5′ sequences in the δ-crystallin mRNA of the duck did not hybridize with the putative leader sequence (exon 1) of the cloned δ-crystallin gene from the chicken. The average lengths and relative positions of all the hybridized and nonhybridized regions of this chicken library clone were similar in the heteroduplexes formed with the duck or the chicken δ-crystallin mRNAs (Figure 6B).

Discussion

The present results indicate that duck δ-crystallin is composed of at least four polypeptides that can be separated on the basis of molecular weight. All four polypeptides resolved here are considered to be δ-crystallin on the basis of their similarity in electrophoretic behavior, their cross-reactivity with anti-chicken δ-crystallin antiserum, and their characteristic change in their ratio of synthesis in the cultured lens treated with ouabain. It is unlikely that the immunoprecipitation of the 49K and 50K polypeptides with the anti-chicken δ-crystallin antiserum was due to a contaminating non-δ-crystallin antibody in the antiserum, since the δ-crystallin antigen was purified by isoelectric focusing of the 200K native protein and not by elution of the polypeptides from the $NaDodSO_4$–urea–polyacrylamide gel. Experiments utilizing urea–polyacrylamide gels have provided evidence that there is further charge heterogeneity among the δ-crystallin polypeptides in ducks and chickens (Clayton, 1969).

The present data indicate that there is a great deal of similarity between the δ-crystallin systems of the chicken and the duck. Instead of a mino 50K and a major 48K δ-crystallin band as found in the chicken (Reszelbach et al., 1977), the duck has minor 50K and 49K bands and major 48K and 47K δ-crystallin bands in $NaDodSO_4$–urea–polyacrylamide gels.

Both the duck (present study) and chicken (Reszelbach et al., 1977) δ-crystallin polypeptides are synthesized in a reticulocyte lysate supplemented with δ-crystallin mRNA, suggesting that the polypeptides with different molecular weights are encoded by separate mRNAs.

The preferential reduction in the synthesis of the lower molecular weight δ-crystallin polypeptides in the embryonic duck lens treated with ouabain is analogous to that observed in the embryonic chicken lens cultured without the vitreous body (Piatigorsky & Shinohara, 1977) or in the presence of ouabain (Shinohara & Piatigorsky, 1977). In the chicken, this effect has been correlated with an increase in the intracellular concentration of Na^+ and Cl^- and a decrease in the intracellular concentration of K^+ (Shinohara & Piatigorsky, 1977, 1980). The mechanism of this ionic control of δ-crystallin synthesis is not known, but cell-free experiments suggest that it is regulated at the level of translation (Shinohara & Piatigorsky, 1980). This is discussed further elsewhere (Piatigorsky, 1980). The striking similarity in the effect of ouabain on the ratio of synthesis of the δ-crystallin polypeptides in embryonic duck and chicken lenses suggests evolutionarily conserved structural features in their mRNAs. This is supported by the extensive homology between the duck and chicken δ-crystallin mRNAs described in the present investigation.

Evolutionary studies on α-crystallin have indicated that crystallins are highly conserved proteins (de Jong et al., 1973). It has been estimated that the amino acid sequence of the αA chain of α-crystallin has undergone an average change of approximately 3% per 100 millon years (de Jong et al., 1975, 1980). This is similar to cytochrome *c*, approximately 7 times slower than globin, and about 20 times slower than the immunoglobulins (Wilson et al., 1977). Much less is known about the evolution of δ-crystallin since it has not been sequenced. The native and subunit molecular weights of δ-crystallins have been maintained during evolution of the reptiles and the birds (Rabaey et al., 1969; Williams & Piatigorsky, 1979a; de Jong et al., 1981). Moreover, circular dichroism and fluorescence studies have indicated that the secondary and tertiary structures of δ-crystallins, in particular the microenvironments surrounding the tryptophan residues, have been preserved during evolution (Horwitz & Piatigorksy, 1980). Although it is not possible to quantify precisely the extent of homology in the base sequences of chicken and duck δ-crystallin mRNAs from the hybridization tests performed in the present experiments, it appears that they are very similar. Except for the putative leader sequence, there appears to be extensive homology throughout the δ-–crystallin mRNAs of the duck and the chicken, as judged by the heteroduplex data. Since ducks and chickens have diverged approximately 100 ± 10 million years ago [based on DNA–DNA annealing experiments, Dr. C. G. Sibley, Museum of Natural History, Yale University, and on fossil evidence, Dr. S. Olson, Smithsonian Institute, National Museum of Natural History, Division of Birds, Washington, D. C. (unpublished experiments)], it appears as if avian δ-crystallin has been highly conserved. It is important to note, however, that a recent investigation comparing tryptic peptides and amino acid compositions of δ-crystallin from the chicken and the tegu has provided evidence that this protein has evolved 2–4 times faster than α-crystallin (de Jong et al., 1981). Furthermore, these authors have suggested on the basis of amino acid composition data that δ-crystallin may be changing faster than any of the other lens crystallins. More definitive information concerning the rate of evolution of this interesting protein awaits amino acid and nucleotide sequencing data.

Acknowledgments

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