

Effects of age and genetic growth rate on the crystallin composition of the chick lens

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ABSTRACT This two part study continues and extends our examination of the effects of age and of genetically determined intrinsic growth rate on the overall protein composition of chick lens epithelia, lens fiber masses and whole lenses. Water-soluble proteins were analyzed by SDS-polyacrylamide and 2-dimensional gel electrophoresis. First, detailed age-related changes in protein expression between day 4 of embryonic development and the 8-week adult stage are described for one normal chick strain. Secondly, comparisons are made between day-old post-hatch chicks of four different genotypes: two genetically unrelated chick strains with a high growth rate and propensity for hyperplasia of the lens epithelium and two unrelated slow-growing strains, both with normal lens morphology. We find that the β/δ -crystallin ratio in lens epithelia and fiber masses is higher in both the slow- than in both the fast-growing strains. The data emphasizes the importance of quantitative and non-coordinate changes in crystallin polypeptide expression during lens growth and development, and implicates growth rate as a modifier of the pattern of crystallin expression.

KEY WORDS: chick lens, crystallin, ageing, genetic growth rate

Introduction

Chick lens development is marked by a series of changes in crystallin expression: these include ontogenic changes (δ -crystallin appearing first followed by β -, α - and finally by γ S-crystallins) and quantitative changes including an increase in α -crystallin and notably in β -crystallin relative to δ -crystallin, the loss of δ -crystallin expression in the early adult cortical fibers, and post-translational changes in α A content (Voorter *et al.*, 1987; Wistow and Piatigorsky, 1988; van Rens *et al.*, 1991; Inoue *et al.*, 1992; Piatigorsky, 1992). These changes, which are both spatially and temporally regulated, act to maintain the appropriate refractive property of the lens as it grows by continual recruitment of lens fiber cells from the LE (see Harding and Crabbe, 1984; Sivak *et al.*, 1989).

However, little is known about the overall protein composition of the growing and aging chick lens at the level of individual crystallin polypeptides. Past studies based on SDS-PAGE have examined very restricted periods of development, and have generally failed to resolve simultaneously all the polypeptide members of all three major crystallin classes (α , β and δ), and most previous studies have analyzed proteins in the presence of urea which can generate artefactual heterogeneity among polypeptides through carbamylation (De Pomerai *et al.*, 1978; Thomson *et al.*, 1978; Ostrer *et al.*, 1981; Bagchi *et al.*, 1982; Pal and Modak 1984). Furthermore, these studies have largely concentrated on synthetic changes, but the existence of post-transcriptional differentials do

not permit extrapolation to the actual levels of the various crystallin polypeptides in functioning fibers, yet these are relevant to the optical properties of the lens and must therefore be the focus of adaptive evolutionary pressure. Using high resolution SDS-PAGE we described changes in the overall protein composition of chick lenses during early post-hatch development (Patek and Clayton, 1985). Here, using both one and 2-D gel electrophoresis, the study is extended to include changes from day 4 of embryonic development through to the 8-week adult stage. This continuous record is intended to provide a baseline for studies in lens aging, and in the response of the lens to conditions affecting growth rate, to genetic variations, and to various pathologies, or for comparison with ontogenic changes in the composition of lens fibers transdifferentiated from different extra-lenticular sources or at different stages of development. To date the analysis of post-hatch chick LFM by SDS-PAGE has revealed striking similarities between the protein composition of lens fibers formed *in vivo* and *in vitro* (Patek and Clayton 1985, 1986, 1988; Patek *et al.*, 1986; Clayton *et al.*, 1991).

While the factors responsible for regulating the various developmental changes in crystallin expression are still poorly understood there is evidence, partly based on analysis of mutant chick strains,

Abbreviations used in this paper: IEF, isoelectric focusing; LE, lens epithelium; LFM, lens fiber mass; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; 2-D, two-dimensional.

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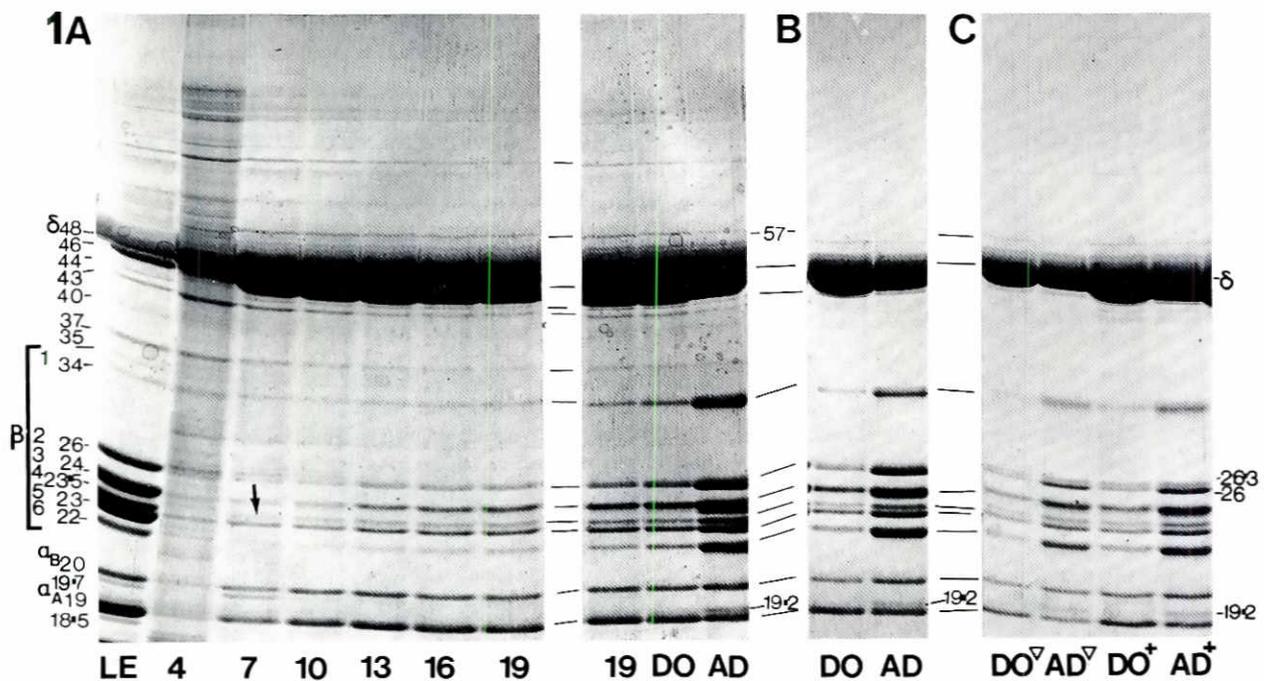


Fig. 1. SDS-PAGE showing developmental changes in water-soluble protein accumulation in chick lens tissues from the normal N-J strain. Tissues include day-old post-hatch LE, 4-day embryonic lenses (4) and LFM from embryonic (days 7, 10, 13, 16 and 19 of incubation), day-old post-hatch (DO) and 8-week adult (AD) chicks. **(A)** 100 μ g protein per lane; **(B)** 60 μ g protein per lane; **(C)** ∇ 30 μ g and ∇ 60 μ g protein per lane. Molecular sizes are indicated in kilodaltons (kDa). As reported previously (Patek and Clayton, 1985) nine crystallin polypeptides were routinely resolved in day-old post-hatch LFM: δ (48 kDa), β_1 (34 kDa), β_2 (26 kDa), β_3 (24 kDa), β_4 (23.5 kDa), β_5 (23 kDa), β_6 (22 kDa), α_B (20 kDa) and α_A (19 kDa). The 19.2 kDa polypeptide detected in adult LFM corresponds to the polypeptide previously designated as either α_A 149-Asp (Voorter et al., 1987) or α_A (Inoue et al., 1992). The 46 kDa polypeptide is antigenically distinct from δ -crystallin (Patek and Clayton, 1985) and is probably τ -crystallin since as reported by Rudner et al. (1990) it represents 5-9% of the water-soluble protein in LE but is present at only trace levels in the lens fibers. Under conditions of high current density (70 mA in C versus 40 mA in A and B) two polypeptides, 26 kDa and 26.3 kDa, could be resolved in the β_2 region which correspond to β_2A and β_2B respectively in 2-D gels (see Fig. 3). δ -crystallin was the most abundant crystallin class in embryonic and day-old post-hatch LFM but β -crystallin was the more abundant in adult LFM. Embryonic LFM contained more α - than β -crystallin but the converse was the case in post-hatch stages. Day-old post-hatch LE was extremely rich in β -crystallin but contained only trace amounts of β_1 . In Fig. 1A the 18.5 kDa polypeptide is detected in LE and 4-day lenses only, the 19.7 kDa polypeptide is detected in LE, 4-day lenses (at trace levels only) and in 7-day LFM only, and the 26.3 kDa polypeptide is detected in 4-day lenses and in 7- and 10-day LFM only. β_4 was absent in 4-day lenses but was detected in 7-day LFM (arrow) onwards.

which implicates the cell cycle. Hy-1 and Hy-2 are genetically unrelated chick strains rigorously selected for high early growth rate. Both exhibit hyperplasia of the LE which is very marked in day-old post-hatch chicks (Clayton, 1975, 1979; McDevitt and Clayton 1979; Randall et al., 1979). Hy-1 and Hy-2 lens cells exhibit a higher rate of mitotic activity *in vivo* and *in vitro* than cells from two genetically unrelated slow-growing inbred strains, N-J (previously referred to as strain N) and N-Rd with normal lens morphologies (see Clayton, 1979; Randall et al., 1979; Patek and Clayton 1986, 1988). Reports that the β/δ ratio is higher in both freshly excised LE and LE cell cultures derived from day-old post-hatch chicks of the N-J than Hy-1 strain (De Pomerai et al., 1977, 1978) suggest that a short mitotic cycle favors δ -crystallin expression while a longer cycle favors β -crystallin (Clayton, 1979). However, the evaluation of the data is affected by several factors. Firstly, the *in vitro* data could be complicated by cell selection since chick LE is heterogeneous for δ -crystallin expression *in vitro*, and Hy-1 cells are very heterogeneous in their capacity for lens fiber differentiation and growth rate, and show poor plating efficiency as compared with N-J cells (see Clayton, 1979; Patek et al., 1986). Secondly, culture age is an important consideration since we now know that after an initial increase, the level of δ -crystallin actually falls in differentiated primary cultures while β -crystallins continue to rise (Patek and

Clayton, 1985). Thirdly, our original *in vivo* data was based on hemagglutination inhibition analysis using polyclonal β -crystallin antisera and it is not clear which β -crystallin polypeptides were affected, and since the affinities of the antibodies used differed between the β -crystallin polypeptides some skewing of the data might have occurred if there were a very large difference in the relative proportions of the β -polypeptides between strains. Finally, since only two strains were compared then it was open to argument that the difference was strain-specific and not meaningfully related to the growth rate. Here we address the issue using SDS-PAGE and by examining freshly excised day-old post-hatch LE from four unrelated chick strains, fast- (Hy-1 and Hy-2) and slow-growing (N-J and N-Rd), in order to assess the effects on the overall crystallin polypeptide composition and determine whether there is a general relationship between mitotic rate and the β/δ -crystallin ratio. For the first time the effect on the crystallin composition of the corresponding lens fibers was also investigated.

Results

Age-related changes

The protein composition of LE and LFM resolved by SDS-PAGE, and of whole lenses resolved by 2-D gel electrophoresis are

shown in Figures 1-3. The resolution of the α -, β - and δ -crystallin polypeptides by SDS-PAGE and their immunological identification in day-old post-hatch chicks has been described (Patek and Clayton, 1985; Head *et al.*, 1991). The 2-D gel profiles are in general agreement with earlier reports (Voorter *et al.*, 1987; Rudner *et al.*, 1990; Head *et al.*, 1991; Inoue *et al.*, 1992). The study does not deal with γ s-crystallin since it is not expressed during embryonic development and comprises <1% of the total protein content of the early adult chick lens (see van Rens *et al.*, 1991).

Numerous qualitative and quantitative changes in polypeptide expression occurred during lens development but only the major changes are detailed below. In relative terms, the levels of each of the α - and β -crystallin polypeptides increased throughout development but the amount of δ -crystallin rose initially between days 4 and 10 and then fell between the day-old post-hatch and 8-week adult stages (Figs. 1 and 2). All three polypeptides resolved in the δ -crystallin region (48 kDa, 50 kDa and 52 kDa) showed parallel changes. Thus a significant increase in the β/δ -crystallin ratio occurs in LFM as early as days 10-13 of development, and

metabolic labeling studies find that the level of δ -crystallin synthesis falls in whole lenses between days 13-16 of development while α - and β -crystallins continue to rise (Fig. 4). τ A and τ B were both detected in 7-day embryonic lenses (Fig. 5A). At this stage τ -crystallin was more abundant than α B and β -crystallin (only β 5 detected) but less abundant than α A and δ -crystallin. τ -crystallin became the least abundant class of crystallin in day-old post-hatch lenses (Fig. 5C). β ₄ was not detected in 4-day embryonic lenses analyzed by SDS-PAGE but all major α -, β - and δ -crystallin polypeptides were present in LFM from at least day 7 (Fig. 1A). In contrast, β ₅ was the only β -crystallin detected in 7-day embryonic lenses analyzed by 2-D gel electrophoresis (Fig. 5A). This discrepancy between SDS-PAGE and 2-D gels presumably reflects the different protein loadings, 100 μ g per lane in SDS-PAGE gels versus 150 μ g total protein in 2-D gels.

Changes in the proportions of the α - and β -crystallin polypeptides observed in both SDS-PAGE and 2-D gels included an increase in the amount of α B relative to α A, but α A remained the most abundant throughout, and a shift from a high abundance of β ₅ to β ₃

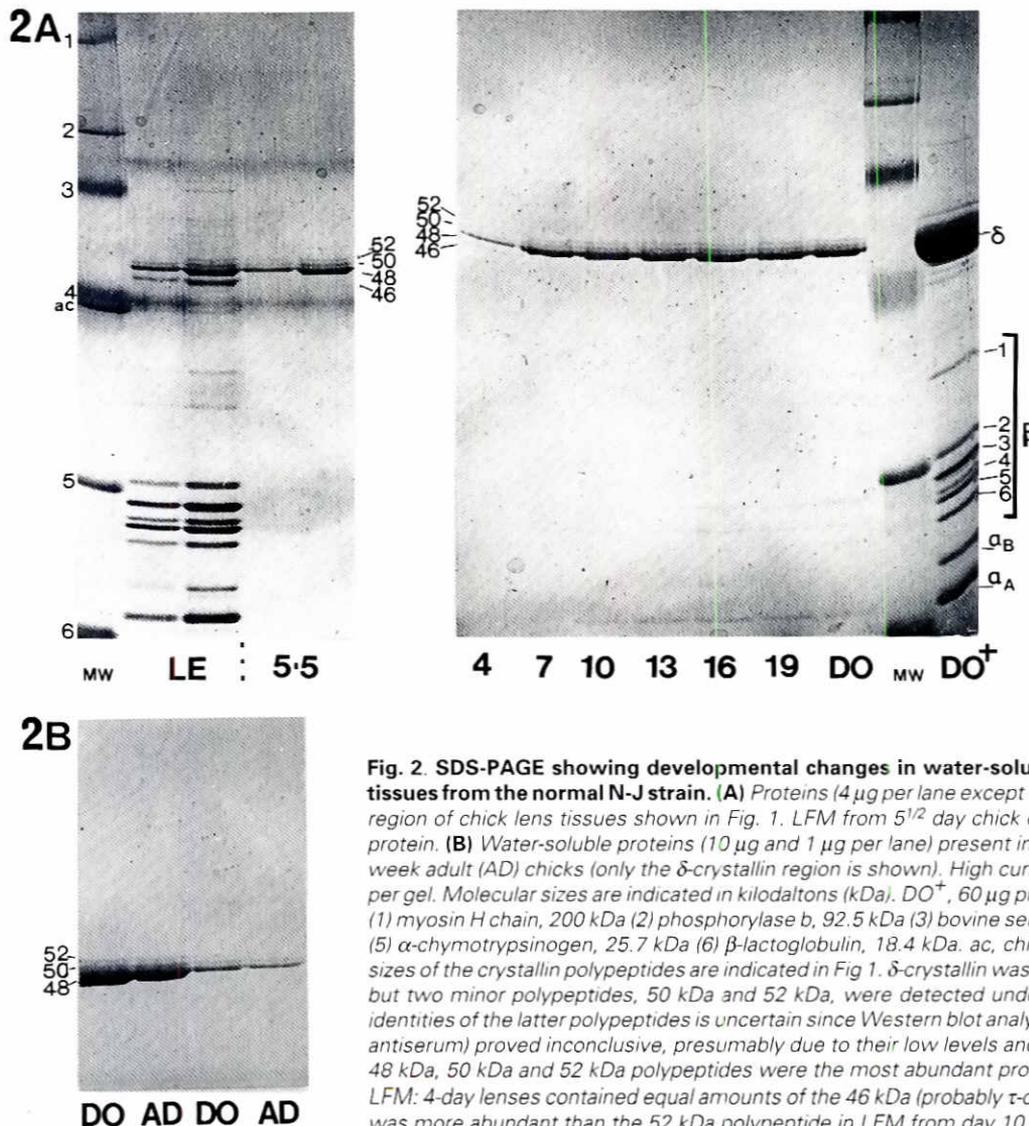


Fig. 2. SDS-PAGE showing developmental changes in water-soluble protein accumulation in chick lens tissues from the normal N-J strain. (A) Proteins (4 μ g per lane except for LE, 20-40 μ g) resolved in the δ -crystallin region of chick lens tissues shown in Fig. 1. LFM from 5^{1/2} day chick embryos are included (5.5). 2 μ g and 4 μ g protein. **(B)** Water-soluble proteins (10 μ g and 1 μ g per lane) present in LFM from day-old post-hatch (DO) and 8-week adult (AD) chicks (only the δ -crystallin region is shown). High current density conditions were used, 70 mA per gel. Molecular sizes are indicated in kilodaltons (kDa). DO⁺, 60 μ g protein; mw, molecular weight size markers (1) myosin H chain, 200 kDa (2) phosphorylase b, 92.5 kDa (3) bovine serum albumin, 68 kDa (4) ovalbumin, 43 kDa (5) α -chymotrypsinogen, 25.7 kDa (6) β -lactoglobulin, 18.4 kDa. ac, chick muscle G-actin, 43 kDa. The molecular sizes of the crystallin polypeptides are indicated in Fig 1. δ -crystallin was routinely resolved as a 48 kDa polypeptide but two minor polypeptides, 50 kDa and 52 kDa, were detected under conditions of high current density. The identities of the latter polypeptides is uncertain since Western blot analysis (using a monospecific chick δ -crystallin antiserum) proved inconclusive, presumably due to their low levels and masking by the 48 kDa polypeptide. The 48 kDa, 50 kDa and 52 kDa polypeptides were the most abundant products present in 5^{1/2} and 7-day embryonic LFM: 4-day lenses contained equal amounts of the 46 kDa (probably τ -crystallin) and 50 kDa polypeptides, and α A was more abundant than the 52 kDa polypeptide in LFM from day 10 of development.

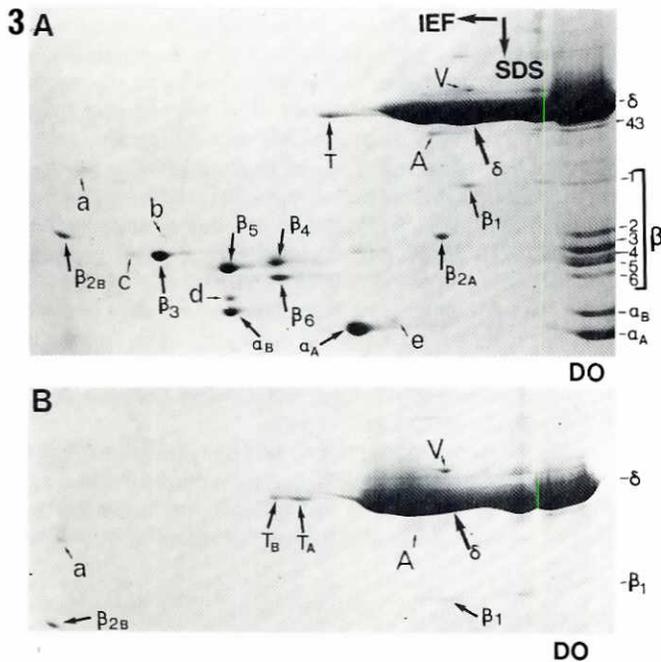


Fig. 3 2-D gel electrophoresis showing water-soluble proteins present in day-old post-hatch chick lenses from the normal N-J strain. Molecular sizes are indicated in kilodaltons (kDa). Separation was by IEF in the first dimension followed by SDS-PAGE (SDS), 150 μ g protein per gel. Orientation at the IEF arrow denotes the acidic to basic pH gradient. DO, reference lane showing proteins present in LFM from day-old post-hatch chicks resolved by SDS-PAGE. δ -crystallin was resolved as a broad band (pI 3.5-6.1) resulting from protein overloading and possibly also reflecting the mixture of oligomeric species, mainly dimers, due to incomplete dissociation of δ -crystallin (see Piatigorsky, 1984). Seven β -crystallin polypeptides were resolved: β_1 (34 kDa, pI 4.9), β_2 (24 kDa, pI 6.8), β_3 (23.5 kDa, pI 6.2), β_4 (23 kDa, pI 6.5), β_5 (23 kDa, pI 6.5), β_6 (22 kDa, pI 6.2) and two in the β_2 region designated β_{2B} (pI 9.0) and β_{2A} (pI 5.0). On the basis of relative abundance the β_{2A} and β_{2B} polypeptides probably correspond to the 26 kDa and 26.3 kDa polypeptides respectively which could be resolved by SDS-PAGE in the β_2 region under conditions of high current density (Fig. 1C). α -crystallin was resolved as α_A (19 kDa, pI 5.9) and α_B (20 kDa, pI 6.5). Under optimal conditions (see B) τ -crystallin (46 kDa) was resolved as two spots τ_A (pI 6.1) and τ_B (pI 6.2) as reported by Rudner et al. (1990). Spot e (19.2 kDa, pI 5.6) corresponds to the 19.2 kDa polypeptide detected in adult LFM (Fig. 1) and is probably α_A 149-Asp (Voorter et al., 1987). This component was not detected by SDS-PAGE in day-old post-hatch LFM but was detected at trace amounts in some day-old post-hatch lenses (Fig. 5C). As well as actin (A, 43 kDa, pI 5.5) and vimentin (V, 57 kDa, pI 4.1), four products labelled a-d (a; 38 kDa, pI 8.2; b; 26 kDa, pI 6.8; c; 24 kDa, pI 7.1; d; 21 kDa, pI 6.5) were routinely detected. Spots a and b were only evident at high protein loadings (150 μ g in Fig 3A versus 100 μ g in Fig. 5c). Spot a shares similar size and pI values with duck and swan ϵ -crystallin (37-38 kDa, pI 7-8; see Stapel et al., 1985; Wistow and Piatigorsky, 1988). The molecular sizes of the crystallin polypeptides are indicated in Fig. 1.

and β_5 between days 10-13, to a shift to a high abundance of β_3 and β_6 by the 8-week adult stage (Figs. 1A and 5A and B). The latter changes in post-hatch LFM were more apparent at lower protein loadings (Fig. 1B). β_6 was the least abundant β -crystallin in the embryo whereas β_{2B} (26.3 kDa) and β_4 were least abundant in post-hatch LFM and lenses (Figs. 1 and 5C). Development was also marked by an increase in the τ_A/τ_B ratio but τ_A was predominant in both 7-day embryonic and day-old post-hatch lenses (Fig. 5A and C). In contrast, the ratio of the 48 kDa, 50 kDa and 52 kDa

polypeptides resolved in the δ -crystallin region remained unchanged at 10:1:0.5 during development (Fig. 2).

The patterns of accumulated protein in both SDS-PAGE and 2-D gels generally reflected those of protein synthesis (Figs. 4 and 5). However this correlation was not absolute; for example in 2-D gels β_3 was synthesized but not accumulated in 7-day lenses, β_4 showed high synthesis but low accumulation in day-old post-hatch lenses, and the intense synthesis of α_A over α_B was not matched by accumulated levels at either of the developmental stages examined. Finally, the appearance of spot e (19.2 kDa, pI 5.6) in post-hatch lenses did not correspond to any component in either the 2-D gel or SDS-PAGE synthetic profiles (Fig. 5C and D, see 2-D gels and SDS-PAGE reference marker lanes). The latter polypeptide, which corresponds to the 19.2 kDa polypeptide detected in adult LFM by SDS-PAGE, is therefore not a primary gene product, and it corresponds to the age-related post-translational product, α_A 149-Asp, derived from α_A by deamination, (Voorter et al., 1987), and more recently referred to as α_{A_A} (Inoue et al., 1992). We confirm reports by both these groups that this component first appears in day-old post-hatch chicks, and increases in abundance thereafter (Figs. 1 and 5).

Effect of genetically determined growth rate

All four strains (Hy-1, Hy-2, N-Rd and N-J) were very similar in the crystallin polypeptides present and their relative abundance in LE and LFM (Fig. 6). The only qualitative difference was the presence of the 19.7 kDa polypeptide in LE of the slow-growing

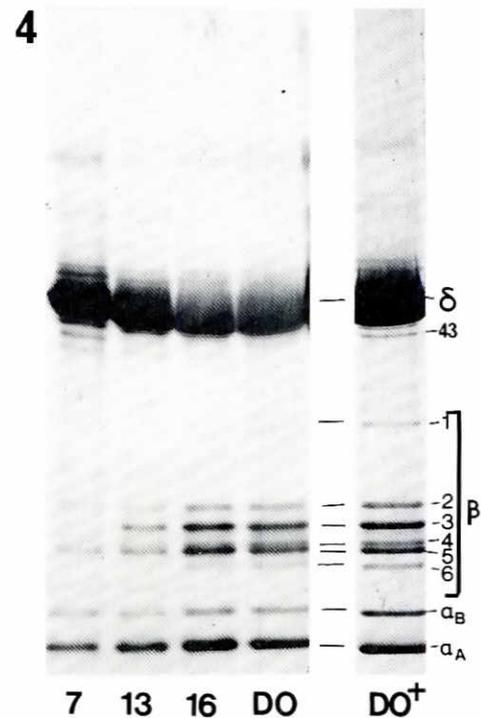


Fig. 4 SDS-PAGE showing developmental changes in water-soluble protein synthesis by chick lenses from the normal N-J strain. 1×10^3 dpm per lane synthesized from ^{14}C -mixed amino acids (0.18 MBq/ml/3h). Tissues include embryonic (days 7, 13 and 16 of incubation) and day-old post-hatch (DO) chicks. DO⁺, reference marker lane (60 μ g protein) stained with Coomassie Brilliant Blue-R. The molecular sizes of the crystallin polypeptides are indicated in Fig. 1.

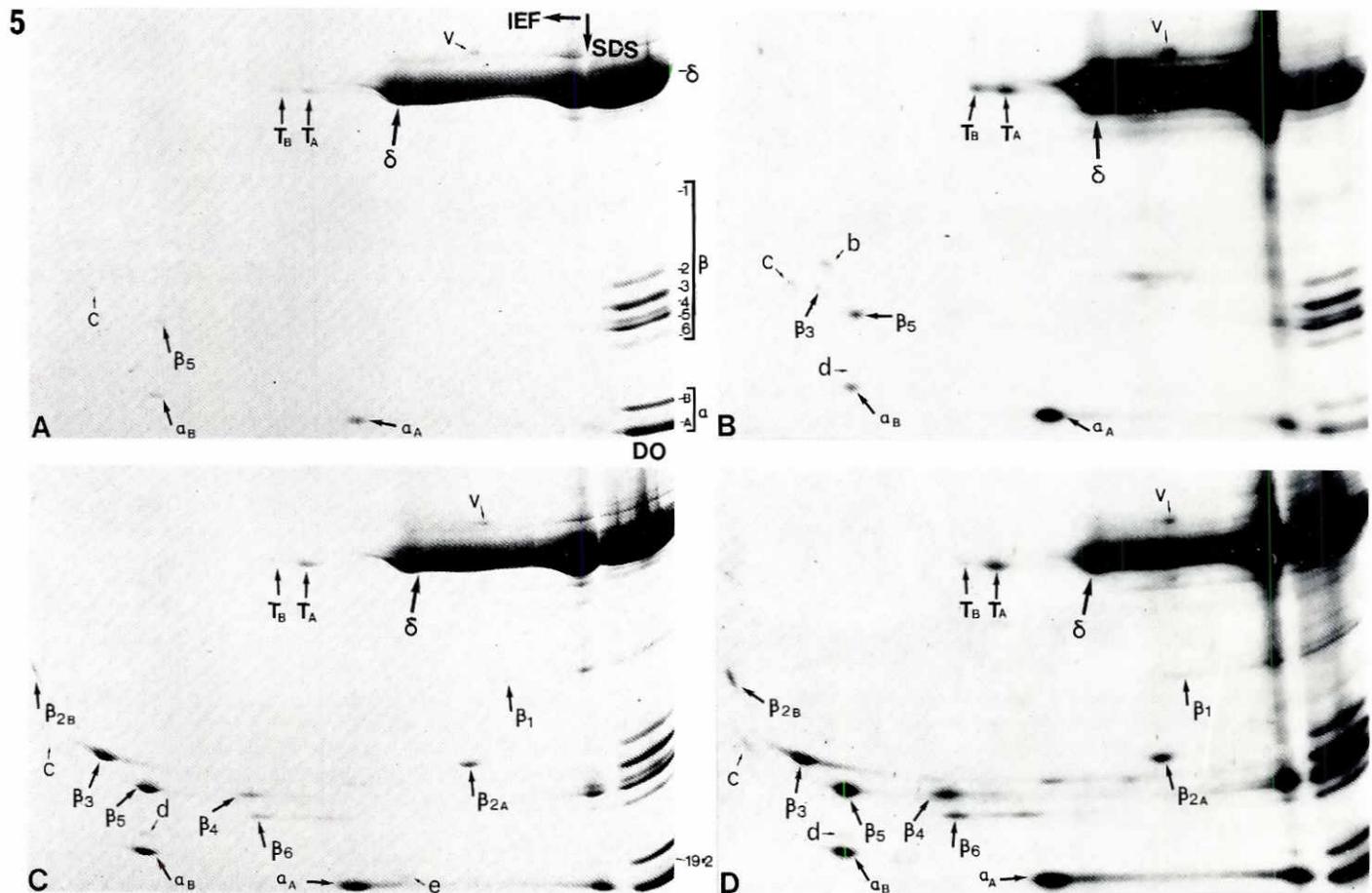


Fig. 5. 2-D gel electrophoresis showing water-soluble proteins expressed by 7-day embryonic (A and B) and day-old post-hatch (C and D) chick lenses from the normal N-J strain. (A,C) Accumulated protein (100 µg per gel); (B,D), synthesized protein from ^{14}C -mixed amino acids (0.18 MBq/ml/3h). Molecular sizes are indicated in kilodaltons (kDa). V, vimentin. A reference marker lane showing day-old chick lens protein (DO), resolved by SDS-PAGE, is included at the extreme right of each gel. Spots a and b are not detected at this lower protein loading. (cf Fig. 3, 150 µg protein). The molecular sizes of the crystallin polypeptides are indicated in Fig. 1. Spot e corresponds to the polypeptide previously designated as either α_A 149-Asp or α_A (see text). δ -crystallin was the predominant crystallin class expressed by 7-day embryonic and day-old post-hatch lenses. 7-day lenses expressed more α - than β -crystallin but the converse was the case in day-old post-hatch lenses.

strains (Fig. 6 lanes A-G). However there were several strain-specific quantitative differences. In general, the β/δ -crystallin ratio was higher in lens tissues from both the slow-growing compared to both fast-growing strains.

These quantitative differences between strains, although small, were confirmed by densitometry and were consistent between all the analytic studies made of numerous separate preparations. LE and LFM from both the fast-growing strains contained relatively more δ -crystallin than both the slow-growing strains, and the minor 50 kDa and 52 kDa polypeptides which could be resolved in the δ -crystallin region, were, like the 48 kDa polypeptide, also more abundant in LFM from Hy-1 than N-Rd (Fig. 6 lanes O and P). The relative contributions of the β -crystallin polypeptides varied both between strains and between LE and LFM of each strain. In LE, β_6 in N-Rd and β_2 and β_6 in N-J were more abundant than in the fast-growing strains. In LFM, β_3 and β_6 in N-Rd and β_{3-6} in N-J were more abundant than in the fast-growing strains. In contrast, the levels of α -crystallin and the non-crystallin polypeptides including actin (43 kDa) and vimentin (57 kDa) remained relatively unaffected in both LE and LFM.

Discussion

We report here for the first time on changes in the overall protein composition of chick LFM throughout embryonic development and address the issue concerning the relationship between growth rate and crystallin expression.

Brahma and van der Starre (1976) reported that the β/δ -crystallin ratio remains unchanged in the chick lens between days 6 and 16 of development but we found that the β/δ -crystallin ratio increases very significantly from at least day 13 of development, in terms of both accumulated and synthesized protein, and that the increase in α - and β -crystallins relative to τ - and δ -crystallins involves all the polypeptide members differentially. While δ -crystallin was the predominant crystallin class in both embryonic and day-old post-hatch lenses, τ -crystallin was more abundant than β -crystallin in 7-day embryonic lenses. Thus τ -crystallin is an unexpectedly abundant component of the early embryonic chick lens. β_4 was not detected in 4-day embryonic lenses but all eleven major crystallin polypeptides [α_A , α_B , τ_A , τ_B , δ (48 kDa) and β_{1-6}] were represented in LFM and whole lenses by at least day 7 of development, at which

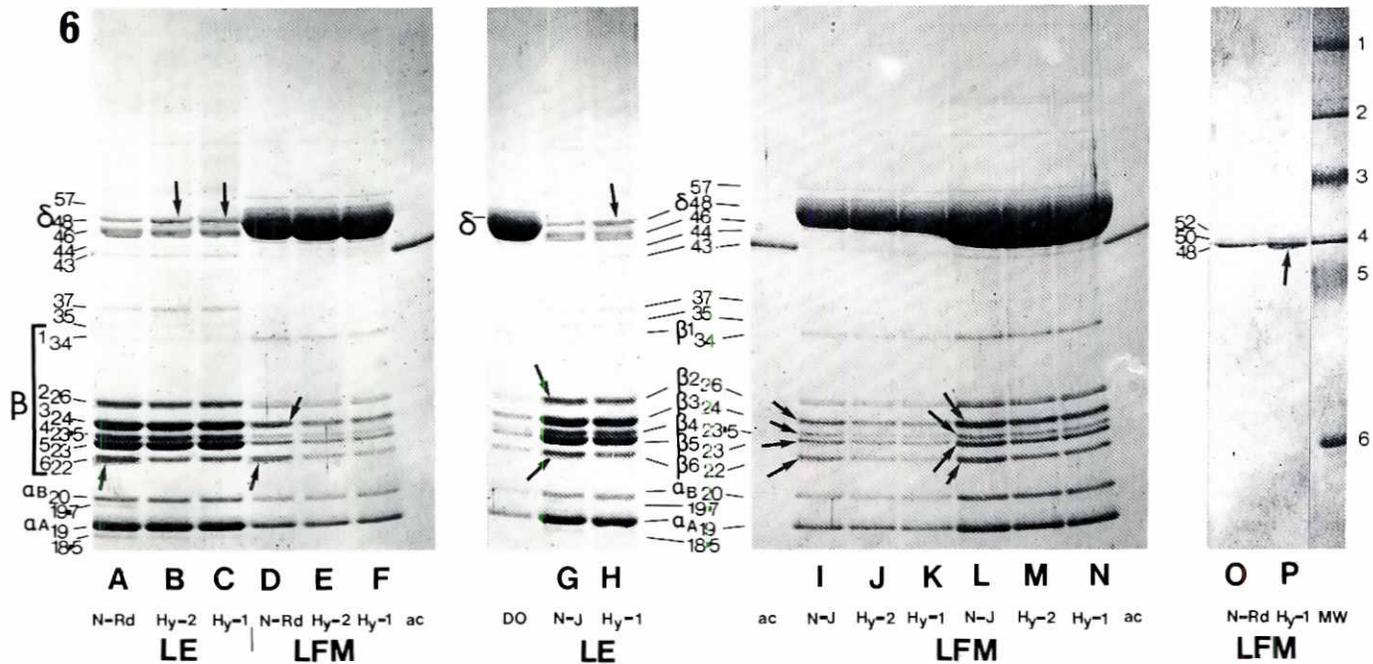


Fig. 6. SDS-PAGE showing water-soluble proteins present in LE and LFM from day-old post-hatch chicks of slow-growing (N-J, N-Rd) and selected fast-growing (Hy-1, Hy-2) strains. All lanes contain 60 μ g protein except for lanes L-N (120 μ g) and O and P (4 μ g). Molecular sizes are indicated in kilodaltons (kDa). DO, LFM from day-old post-hatch N-Rd chicks; ac, chick muscle G-actin (43 kDa); MW, molecular weight size markers as Fig. 2. In each gel, the strain(s) containing the higher level of a particular polypeptide is indicated by an arrow. Due to protein overloading in LFM it was only possible to assess quantitative differences in δ -crystallin in lanes O and P. All three 48 kDa, 50 kDa and 52 kDa polypeptides resolved in the δ -crystallin region were more abundant in Hy-1 than N-Rd and their ratio remained at 10:1:0.5.

time the chick lens becomes uniformly transparent (see Harding and Crabbe, 1984; Stirling and Wakely, 1987). It remains to be determined whether these events are causally related. Secondary lens fiber production commences between days 3 $\frac{1}{2}$ and 4 of development in the chick (see Stirling and Wakely, 1987). Thus the absence of β_4 in 4-day lenses may not only point to selective regulation within a class of related polypeptides but may indicate that β_4 is predominantly or exclusively expressed in secondary fibers. There is other evidence that β -crystallin polypeptides are selectively expressed in different compartments of the chick lens; for example βB_1 , which is linked with the process of fiber differentiation (Ostrer *et al.*, 1981), is expressed in lens fibers before day 18 of development, and thereafter in LE but only in the annular pad (Brahma, 1988).

Lens development was also marked by an increase in the amount of τA relative to τB between the 7-day embryonic and day-old post-hatch stage. If this change continues during post-hatch development then this would account for the report that there is only one isoform of τ -crystallin in adult chick lens fibers (Rudner *et al.*, 1990). Other changes included an increase in the amount of αB relative to αA and a shift from a high abundance of β_5 in early embryonic LFM and lenses to high levels of β_3 and β_6 in adult tissues. Thus αA , β_5 and τB , like δ -crystallin, may be regarded as largely embryonic and possibly nuclear components while αB , β_3 , β_6 and τA may be regarded as predominantly adult and possibly cortical products. However it is difficult to speculate on their precise spatial expression since nuclear fibers, in aged mammalian lenses at least, still retain some capacity for protein synthesis (see Lieska *et al.*, 1992). The 19.2 kDa polypeptide (spot e, pl 5.6) detected in post-hatch LFM and lenses, which here we directly

show is not a primary gene product, is probably formed in the lens nucleus since it corresponds to αA 149-Asp which is derived from αA by age-related post-translational modification (Voorter *et al.*, 1987).

The number of δ -crystallin polypeptides in the chick lens is presently unclear since the number observed varies with the electrophoretic technique, as well as the use of urea (Reszelbach *et al.*, 1977; Thomson *et al.*, 1978; Pal and Modak, 1984; see Piatigorsky, 1984). Using SDS-PAGE Reszelbach *et al.* (1977) resolved two δ -crystallin polypeptides in the 15-day embryonic chick lens, 48 kDa and 50 kDa (in the ratio 3:1), but only in the presence of urea. In the present study three polypeptides, 48 kDa, 50 kDa and 52 kDa, were resolved in the δ -crystallin region by SDS-PAGE in the absence of urea. Although they were the most abundant products present in δ -crystallin-rich 5 $\frac{1}{2}$ - and 7-day embryonic LFM, the identities of the 50 kDa and 52 kDa polypeptides is uncertain. However their ratio remained fixed throughout development (10:1:0.5) suggesting that their expression might be regulated by a shared mechanism. If all three are δ -crystallin polypeptides then it would appear that the ratio of the δ -crystallin polypeptides, unlike that of the α -, β - and τ -crystallin polypeptides, is unaffected by development and aging.

Li and Beebe (1991) reported that crystallin mRNA stability did not change during development, and the strong correlation found here between crystallin polypeptide accumulation and synthesis supports the view that there is little post-transcriptional turnover in the lens (see McDermott *et al.*, 1992). However we found that this correlation was not absolute, indicating that differential turnover of some polypeptides, including αA , β_3 and β_4 , appears to occur over a limited period of development.

The genetic study, based on four unrelated chick strains each with different intrinsic growth rates of the LE, found that strain differences only involved δ -crystallin and some of the β -crystallins, and that the β/δ -crystallin ratio was higher overall in LE from the slow- than the fast-growing strains. The results support the view that crystallin expression is affected by growth rate, with a short cell cycle time favoring δ -crystallin expression and a longer interval favoring β -crystallin (Clayton, 1979; see Introduction). This hypothesis is further supported by other independent evidence. Firstly, early embryonic chick LE cells which have a high growth rate, synthesize more δ - than β -crystallin, whereas the converse is true with slower growing cells from later stages of development (see Clayton, 1979; Harding and Crabbe 1984; Clayton *et al.*, 1991). Secondly, agencies which shorten or, conversely, lengthen the mitotic interval of cultured chick lens cells also have such effects on the β/δ -crystallin ratio (reviewed Clayton *et al.*, 1991). Thirdly, the β/δ -crystallin ratio rises more rapidly in long-term LE cultures derived from day-old post-hatch chicks of the two slow-growing strains compared to the two fast-growing strains (Patek and Clayton, 1988). Finally, the report by Sawada *et al.* (1992) that β -crystallin is only expressed by non-dividing chick lens cells *in vivo* and *in vitro* also supports this view. It is significant that the higher β/δ -crystallin ratio in LE of the slow-growing strains was also found in the corresponding LFM which implies that the properties of the post-mitotic fiber cells reflect the epithelial cells from which they are derived. This relationship, which presumably only applies to the recently differentiated cortical fibers, would explain the ontogenic shift from high levels of δ -crystallin to β -crystallin expression in the chick lens which commences on about day 13 when LE mitotic activity is in decline (reviewed Harding and Crabbe, 1984).

The data emphasizes the importance of non-coordinate and quantitative changes in crystallin polypeptide expression both within and between crystallin classes, throughout the post-inductive stages of chick lens development, and implicates the cell cycle as an important factor in this regulation.

Materials and Methods

For aging studies we used the normal N-J strain and compared the composition of 4-day embryo lenses, LFM from day 5^{1/2} of incubation to the 8-week adult, and LE, including most of the annular pad, from day-old post-hatch lenses. Growth rate studies were undertaken by using LE and LFM from day-old post-hatch chicks of the two fast-growing (Hy-1 and Hy-2) and two slow-growing (N-Rd and N-J) strains (see Clayton, 1979; Patek and Clayton, 1986, 1988). In all cases tissues were pooled from at least twelve chicks and stored immediately in liquid N₂ until analyzed.

To assess protein synthesis freshly excised whole lenses were incubated in culture medium (Patek and Clayton, 1985) supplemented with L-[U-¹⁴C] mixed amino acids (0.18 MBq/ml/3h) (Amersham, UK) then stored in liquid N₂ until analyzed. Water-soluble proteins were analyzed within 2 h of extraction to avoid possible artefacts due to degradation. Protein extraction and SDS-PAGE was performed as described previously (Patek and Clayton, 1985) and 2-D gel electrophoresis was by IEF in the first dimension followed by SDS-PAGE (Head *et al.*, 1991). Proteins were stained with Coomassie Brilliant Blue-R, and fluorography and densitometry were performed as before (Patek and Clayton, 1985). Molecular weight size markers and chicken muscle G-actin were purchased from The Sigma Chemical Company, UK.

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