Ontogeny of lens crystallins in marine cephalopods

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SUMMARY

The ontogeny of the lens crystallin antigens throughout development of three marine cephalopod embryos, Loligo vulgaris, Sepia officinalis and Octopus vulgaris has been investigated using the indirect immuno-cytochemical staining against respective homologous anti-total lens protein antiserum.

The cellular mechanism of lens development appears to be the same in all species investigated and so does the ontogeny of the lens crystallins. The first positive immunofluorescence reaction appears simultaneously and in equal intensities over the lens and lentigenic area confirming the relationship between the two.

INTRODUCTION

The cephalopod eye lens is similar to the vertebrate eye lens in function but the development and the lens protein contents of the two are known to be quite different. In vertebrates, the lens develops from the overlying ectoderm in contact with the optic vesicle. In cephalopods, on the other hand, the lens develops from the lentigenic area within the optic vesicle and has been called 'ciliary body' or 'corpus epitheliale' (Williams, 1909). Arnold (1966, 1967) studied the fine structures of the developing lens in Loligo pealii and Octopus vulgaris and he found that the course of lens development in both Loligo and Octopus is quite similar. He used the term 'lentigenic body' in place of ciliary body.

Fine cytoplasmic (lentigenic) processes are given out from the cells of the lentigenic body and these fuse in the posterior chamber of the optic vesicle to form the lens primordium. Growth in such a lens is accompanied by addition of lentigenic processes to its body and these processes do not contain any nuclei. In the vertebrate lens, on the other hand, growth is accompanied by addition of fibre cells from the germinative zone of lens epithelium (Papakonstantinou, 1967). Fibre cell differentiation represents a terminal cellular phase and is accompanied by pycnosis, degeneration and ultimate disappearance of the cell nuclei in a strict temporal and spatial manner (Modak & Perdue, 1970).

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When fully formed, the lens in cephalopod consists of an anterior plano-convex and a posterior sub-spherical section. These two sections develop from the inner and outer cells of the lentigenic body at different times.

The structural proteins or the crystallins in cephalopod lens are immunogenic and have been called α-, β-, γ-, and δ-crystallins similarly as in the fish (Bon, Dohrn & Batnik, 1967).

Immunological investigations have revealed that cephalopod and vertebrate lens antigens do not cross-react and it has been suggested that the biochemical evolution of these analogous structures has followed distinct pathways (Halbert & Manski, 1963).

There are relatively few studies on cephalopod lens protein composition (Clayton, 1974) and to the knowledge of the present author no information relating to the ontogeny of the lens crystallins in cephalopods is available.

This communication reports the ontogeny of the lens crystallins in *Loligo vulgaris*, *Sepia officinalis* and *Octopus vulgaris* during normal development as revealed by the indirect immuno-cytochemical staining method using respective homologous anti-total lens protein antiserum.

**MATERIALS AND METHODS**

**Preparation of antisera**

Lenses were carefully removed from freshly killed *L. vulgaris*, *S. officinalis* and *O. vulgaris* at the Zoological Station, Naples. These were cleaned and homogenized over ice in cooled distilled water using motor driven homogenizer. The homogenates were centrifuged and the supernatants were lyophilized and stored at −20 °C. These lyophilized samples were used in the author's laboratory to raise antibodies in young rabbits according to Rowe (1970). The antisera were tested against their homologous antigens in micro-immunoelectrophoresis and stored at −80 °C for future use.

**Preparation of tissues and tissue sections**

*L. vulgaris*, *S. officinalis* and *O. vulgaris* embryos were collected during the months of May and July/August 1977 at the Zoological Station, Naples. These were staged according to Naef (1923), fixed in cooled 96% ethanol overnight at 4 °C and processed for the immunocytochemical staining according to McDevitt, Meza & Yamada (1969). Serial sections, 6 µm thick, were cut through the eye regions and stored at −20 °C.

The antisera used were diluted 1:4 with 0.01 M phosphate-buffered saline (PBS) at pH 7.0. Fluoresceine isothiocyanate (FITC) conjugated goat-anti-rabbit-γ globulin (Nordic Immunological Laboratories, The Netherlands) were also diluted 1:6 with PBS before the use. Sections were stained using methods already described (Brahma & van Doorenmaalen, 1971).

Two different control series were run simultaneously. In one, the primary
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Fig. 1. Micro-immunoelectrophoresis of (A) *Loligo vulgaris*, (B) *Sepia officinalis* and (C) *Octopus vulgaris* total soluble lens proteins against their respective homologous antiserum. Electrophoresis was performed at 4 °C for 90 min with 250 V and 25 mA current. Immunodiffusion was allowed to continue for 24 h in a humid chamber at the room temperature. After processing the slides were stained with Coomassie brilliant blue R-250. + = anode; − = cathode. The arrow shows the second immunoprecipitin line.

antiserum was replaced by serum from a non-immunized rabbit and in the second, the primary antiserum was omitted.

The sections were examined with a Leitz fluorescence microscope under identical conditions to those previously described (Brahma, Rabaey & van Doorenmaalen, 1972). Photomicrographs were taken with Kodak Tri-X Pan film. Phase-contrast pictures were also made from the same preparations to demonstrate the site of the immunoflorescence.

RESULTS

Immunelectrophoresis of *L. vulgaris*, *S. officinalis* and *O. vulgaris* total soluble lens proteins against their respective homologous antisera are shown in Fig. 1 (A–C). Each of the antigens produced two immunoprecipitin lines on the cathodic side. One is very extensive in each and the second one is weak in *Loligo*, prominent in *Sepia* and strong in *Octopus* (shown by arrow).

Immunohistochemical studies of the lens proteins from these animals (to be reported shortly) showed that the extensive arc in *Loligo* and *Sepia* lens proteins
exhibited reaction of identity when tested against heterologous anti-Sepia and anti-Loligo lens antisera; while neither of these two antisera reacted against Octopus lens proteins. Anti-Octopus lens antiserum, on the other hand, showed reaction of partial identity with the minor component of Sepia lens proteins. However, it showed no reaction at all against Loligo lens proteins.

The present investigation confirms the results of Arnold (1967) that the cellular mechanism of lens development is similar in all the three species. Moreover, it appears that the distribution patterns of the lens crystallins are similar in these three species investigated. This could be due to the presence of the extensive arc in all of them. The following detailed report refers to *L. vulgaris* embryos.

The first positive immunofluorescence reaction was observed at stage 10. In this stage the lens appears as an outgrowth from the lentigenic area. The staining reaction is weak but positive and is localized in the lens as well as in the lentigenic area (Fig. 2). At stages 11 and 12 the lens becomes club-shaped. Its volume is increased and so is its intensity of immunofluorescence (Figs. 3, 4). At stage 13, the lens becomes rounded and the anterior section of the lens begins to appear. The lentigenic area becomes dumb-bell shaped due to its retraction on both sides. There is an overall increase in immunofluorescence both in the lens and in the lentigenic area. Fluorescent cytoplasmic connexions between the lens and the lentigenic area are also visible (Fig. 8). At stage 18, the anterior and posterior sections of the lens are clearly distinguishable and so are their connexions with the lentigenic area. The intensity of immunofluorescence remains unchanged and, as in the previous stages, it is confined to the lens, lentigenic area and the connexions between the two (Fig. 9). At stages later than 18, when the lens is fully developed its anterior and posterior sections are seen lying separated from one another but remain connected with the lentigenic area on either side. The intensity of immunofluorescence and its localization remain as in stage 18 (Fig. 10). Although the anterior section of the lens develops later, the intensity of its immunofluorescence is found to be similar to that of the posterior part.

In every stage investigated, only the lens, lentigenic area and the cytoplasmic connexion between these two showed positive immunofluorescence. The lens and the lentigenic area became fluorescent simultaneously and exhibited equal fluorescence intensities.

In the control series there was no immunofluorescent staining reaction in the absence of primary antiserum.

**DISCUSSION**

According to Arnold (1966, 1967, 1971) proteinaceous materials appear only in the centre of the lens and the microtubules present in the lentigenic processes are somehow connected with the transport of these materials, which ultimately obliterate the lens cytoplasm. Arnold did not observe any such material in the
Figs. 2–4. Dark-field photomicrographs.

Fig. 2. Immunofluorescence photomicrograph of a lens section from stage-10 embryo exposed to *L. vulgaris* anti-total lens protein antiserum. × 360.

Fig. 3. Immunofluorescence photomicrograph of a lens section from stage-11 embryo exposed to *L. vulgaris* anti-total lens protein antiserum. × 360.

Fig. 4. Immunofluorescence photomicrograph of a lens section from stage 12 embryo exposed to *L. vulgaris* anti-total lens protein antiserum. × 360.

Figs. 5–7. Phase-contrast photomicrographs of the above sections with identical magnifications and shown as histological references.
Figs. 8–10. Dark-field photomicrographs.

Fig. 8. Immunofluorescence photomicrograph of a lens section from stage-13 embryo exposed to *L. vulgaris* anti-total lens protein antiserum. × 360.

Fig. 9. Immunofluorescence photomicrograph of a lens section from stage-18 embryo exposed to *L. vulgaris* anti-total lens protein antiserum. × 256.

Fig. 10. Immunofluorescence photomicrograph of a lens section from stage-18 + embryo exposed to *L. vulgaris* anti-total lens protein antiserum. × 224.

Figs. 11–13. Phase-contrast photomicrographs of the above lens sections with identical magnifications excepting Fig. 13 (× 300) and are shown as histological references.
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lentigenic area but recorded a great increase of RNA in this area at the time of appearance of the protein granules in the lens.

The evidence presented here shows clearly that the first positive immunofluorescence reaction to total lens protein antiserum appears simultaneously in the lens and in the lentigenic area. This feature is unique and has never been recorded in vertebrate lens. The fluorescent cytoplasmic connexion between the lentigenic area and the lens is suggestive of transport of lens proteins from the lentigenic area to the lens body.

In cephalopod embryos the lentigenic processes which form the lens do not contain nuclei but they contain polyribosomes (Arnold, 1966, 1967) which may account for the protein synthesis. Protein synthesis in the absence of nucleus has also been recorded in the vertebrate lens fibre region and this is also found to be due to the presence of long-lived m-RNA (Reeder & Bell, 1965; Stewart & Papaconstantinou, 1967).

Only two classes of proteins were detected in the present experiment and as the antibodies were directed against total soluble lens proteins it could not be ascertained which class appeared first during lens development. However, it appears that in all the species investigated the same antigen or antigens found in the lens are also found in the lentigenic area, thus confirming the relationship between these two areas.

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