

A Study on the Embryonic Development of Eye in *Rana Cynophlyctis*



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Abstract

The embryonic development of eye of *Rana cynophlyctis* is controlled by a complex series of interaction in the anterior region of early embryo. In the embryos of external gill stage the optic vesicles come in the contact with head ectoderm. After inductive response the ectodermal cells forming a thickening on each side, the lens placode develops. At a definite external gill stage lens rudiment is evident. Later on lens becomes a vesicular shape. At hind limb bud stage – foot spatula stage lens becomes solid and cellular. Now lens has a crystalline cone. Outer epidermal cornea becomes transparent between the stage 30-33 then inner cornea also appears. Crystalline lens fibers start to differentiate at stage 34. Visual cells of the retina also start to differentiate. All compounds of the eye differentiate at latter stage. In the tadpoles of the *Rana cynophlyctis* eye development appears early as the tadpole at hatching stage swims actively, thus at hatching stage the embryos are not blind. This research work provides preliminary knowledge about Ontogenetic Development of eye and it would be useful in regeneration of eye related studies in Amphibians as well as all vertebrates.

Keywords: Embryonic Development, Optic Vesicles, Ectoderm, Placode, Lens and Eye

Introduction

Morphogenesis of the eye involves a series of co-ordinated sequential changes of inductive interaction between tissues derived from different sources which have been experimentally analysed chiefly in amphibian and chick embryos; but the information gained is apparently applicable to all vertebrates.

Aim of the Study

To get knowledge about embryonic development of eye in *Rana cynophlyctis* is the main aim of the study and this study would be significant for other studies of embryonic development of amphibians as well as in other vertebrates. The study regarding the morphogenesis and differentiation of lens would also be some use for analysis of the mechanism of lens formation from iris under the influence of some chemical such as vitamin A, (during lens regeneration process) in froglet stage and adult stage of *Rana cynophlyctis* and other higher vertebrates.

Review of Literature

These experimental studies have been summarized and reviewed by Twitty (1955) Lopashov and Stroeveva (1961) Lopashov (1963) Coulombr (1961-1965); Reyer (1977) Bard and Ros (1981); Hay (1978) Jacobson and Gordon (1976); Bee (1982).

General accounts of the morphological and histological structure of the anuran eye as a whole and its constituent elements including the retina and visual cells are found in works of many authors (Prince 1956; Nilsson 1964a,b; Lopashov and Stroeveva 1961, Lopashov 1963, Reyer 1977)

Hypothesis

Our study is purely based on laboratory experiments not a hypothetical study.

Materials and Methods

The observation concerning with the ontogenetic development of eye are based mainly on the studies made on the embryos on selected stages. The staging of developing embryos is based on the normal table of *Rana cynophlyctis*, stated by Soni and Sharma. In this normal table the tail bud stage of the embryo is designated as 25-26; "Gill formation stage 27-28-29; Hatching occurs at stage 30; metamorphosis begins at stage 51

begins at stage 51 and the fully metamorphosed froglets is designated as stage 57 after a preliminary examination of developing eyes it has been observed that development of eyes begins at neurula stage and continuous through the larval period and the adult structure is attained after metamorphosis. For the experiment purpose spawn was collected from the pond and reared in the laboratory 30 embryos of different developing state were employed as the embryo shows any change into developing eye the embryos were preserved in Bouin's solutions for the histological purpose. A microscopic camera was also used for taking photography of different developing stages and binocular microscope was also for studying these stages.

Results and Discussions

For the study of ontogenetic development of eye, 5 embryos were preserved at each change. Histological sections were made for detail study. The development of eye if epigenetic occurring in gradual steps over several weeks. It begins at neurula stage of the embryo, continues through the larval period and the adult stage attained after metamorphosis. Stage 25-27: These are the tail bud and gill bud formation stage prior to hatching. At this stage the developing eye is represented by a broad optic vesicle, bulging out laterally from the diencephalon. The outer margin of this vesicle is in contact with the inner layer of head ectoderm (fig-1) By stages the outer wall of this optic vesicle starts thickening and invaginating (fig-). At stage 27 invagination has progressed further and the optic vesicle is assuming the shape of a double walled cup. The inner nervous layer of ectoderm is contact with the optic vesicle has thickened to form the rudiment of lens (fig-1, 3 and 4).

At hatching stage 30 the optic vesicle has now become definitely a double walled cup whose thin outer wall is the future retinal pigmented epithelium (RPE) and thick inner wall the rudiment of normal retina. The lens rudiment has not yet separated completely from the inner layer of ectoderm. It is still solid and thick but curving inwards indicating beginning to the formation of lens vesicle (fig – 4, 5). The rim of the cup now grow out ward, particularly in its ventral and lateral regions, these being the regions which as a result of the direction of invagination, are further from the ectoderm. This outward extension of the sides of the cup, leaves between their ventral edge a slight fissure extending inward to the optic stalk. This is the choroids fissure. At stage 33 the optic cup is still broadly connected with the brain cavity. RPE cells are still rather flat and with out pigment Neural retina is very thick containing densely packed nuclei but there is yet no sign of regionalizations of these nuclei into fixed layers. A space separates the outer wall (RPE) from the inner thicker wall (neural retina) of the optic cup. The lens rudiment has separated from the ectoderm and became vesicular. Lens is located within the edges of the rim of the optic cup (fig 5, 6 & 7) some mesenchymal cells appear to be invading the space between the lens and the corneal ectoderm. This mesoderm later forms the inner cornea.

The cells of the RPE are cuboidal and pigmented but these features are distinctly more pronounced in the dorsal than the ventral retina. A distinct though narrow inner plexiform layer (IPL) separates the ganglion layer (GL) from the inner nuclear layer (INL) in the central part of the neural retina, but the ganglion cells do not yet form a well organized layer. Ora serrata is well indicated marking the dorsal and ventral limits of the developing neural retina and the region which will develop into iris. There is no outer plexiform layer (OPL) separating the inner nuclear layer from the outer nuclear layer (ONL); But the nuclei of ONL appear to be arranged in a single row and small cytoplasmic buds are bulging out from them into the ventricular space towards the PRE indicating the beginning of visual cell differentiation. The lens is still vesicular but its wall become thick crystalline fibers appeared in the elongated cells of the posterior side of the posterior side of the lens (fig- 8, 9 & 10).

At hind limb bud stage the dorsal retina is much larger than the ventral retina. This part shows greater degree of differentiation. The lens becomes solid. At the next stage i.e. stage 34 the degree of development become more advanced. The dorsal retina shows greater degree of differentiation. The vascular choroid is now differentiating out side and around the retina. The cells of retina pigmented epithelium is pigmented but the pigments are denser in the dorsal part. The optic nerve has formed. The nuclei of ONL are arranged in a single layer and visual cell differentiation is more advanced (Fig -11). The pigmented peripheral part of the optic cup distal to ora serrata which forms its rim has turned towards the lens indicating beginning of the formation of iris. The lens is solid with crystalline fibres filling up its interior. The cells of the epithelium around the outer half of the lens are cuboidal and many of them are seen dividing (Fig-11, 12). The corneal epidermis is now becomes transparent. The inner cornea encloses the aqueous chamber. The initial steps in laying down the basic structure of the eye have been completed by this stage. The retina is regionalized, visual cells are differentiating optic nerve is formed, lens is crystalline and cornea is transparent considering that at this stage, the tadpole has started active feeding and prefers lighted conditions to do so, it can be assumed that the eye is now able to perceive the light stimulus. State – 44-50 : The state of development of the eye at stages 45, 47 and 50 is illustrated by section shown in (Fig-12)

Out side the retinal –pigmented epithelium a pigmented as vascular choroid coat has formed it become thicker and more densely pigmented. The eye has grown in size and there is a spacious vitreous chamber. The iris is well formed (Fig- 12). The lens is a soled sphere of crystalline fibers surrounded on there sides by epithelium of cuboidal cells. A spacious aqueous chamber is present bounded externally by a mesodermal inner cornea which is continuous with the mesodermal component of iris. The inner cornea is well separated from the epidermal cornea. The neural retina is well regionalized. The ganglion cells are

arranged in a single row on the inner margin. The ganglion layer (GL) is separated from the inner nuclear layer (INL) by a thick IPL. The INL consists of 2-3 of nuclei and is separated from the ONL by a relatively thin but distinct OPL. The visual cell get differentiated.

The differentiation of these cells and regionalization of neural retina has occurred from the center to the periphery and from inner margin towards the outer margin respectively. Morphogenesis of the eyelids and the cartilaginous scleroid coat begins . Stage 51 -57 at these metamorphosing stages both fore limbs emerge , tail resorption formation of adult type photo rod cell and other metamorphic changes occur .The small tail less froglets hops out of water indicating the end of metamorphosis. The iris attains a high degree of differentiation and becomes, the adult type. The mesodermal inner cornea fuses with the inner layer of corneal epidermis and becomes thick and fibrous. The upper and lower eyelids and nictitating membranes are formed during this early periods of metamorphosis. Sclerotic coat is well formed .Differentiation of this coat also begins around the dorsal retina and spreads to the ventrals nasal and temporal sides. Changes occur in the structure of rods during metamorphosis, and a four cell types appears for the first time during this period .When metamorphosis begins, then onwards the main process in that of growth in size of the eye untills the adult attains its ultimate size.

Similar to other vertebrate the morphogenesis of eye of *Rana cynophlyctis* too, is controlled by a complex series of interactions in the anterior region of early embryos. Shortly after the neurula is formed, the future fore brain region evaginates on left and right sides forming the bulging optic vesicles. These push through the loose mesoderm to the head and make contact with the ectoderm. In response to this contact referred to as inductive response, the ectoderm cells elongate perpendicularly to the zone of contact, forming a thickening on each side, the lens placode. The placode then invaginates and detaches from the overlying ectoderm to form of the lens of the eye as the lens primodium invaginates, the optic vesicles reverse its outward bulge and invaginates, forming the optic cup, The lens placode and optic cup mutually accommodate to each other. The lining of the eye cup subsequently forms the retina. Experimentally it has been examined that the formation of the optic vesicles requires a prior inductive action of the prospective head mesoderm on the anterior portion of the neural plate. In the absence of the induction or in the event that the neural ectoderm is unable to respond, an eyeless condition results .

The literature on lens by the optic vesicle in the amphibians is voluminous the same was also reported in the chick (Waddington and Cohen, 1936 Alexander , 1937 , Lengman , 1959 , and in the mouse (Muthukkaruppan , 1965) . It is true that in normal development of lenses always appear in association with the optic cup. It is also known that the action of the optic cup is essential for the full

realization of growth and differentiation of the lens. Chick lens becomes incomplete after the optic vesicle has been partially extirpated. This incompleteness depending on the amount and quality of material removed (Genis Galve, Santos and Rios (1967). Reyer studied the influence of neural retina and lens on the development of embryonic lens vesicles in *Amblystoma punctatum* and *Triturus Viridescens* and found that lens vesicle of both has the capacity to form lens fibers in an extra ocular environment such as the dorsal fin where it is no longer influenced by the eye cup. There is now considerable evidence that FGF is involved in lens differentiation and growth throughout life. Lovicu et al (1997) concluded that during lens differentiation distinct expression of FGF was associated with elongating Primary fiber cells.

Conclusion

The development of the lens in embryos continues to provide one of the clearest and most classic example of dependent differentiation in which some cells are induced to differentiate into specific organ or tissues by the presence of other cells. Formation of optic vesicle requires an inductive action prospective head mesoderm on the anterior part of neural tube of embryo after it formation the optic vesicle induces the formation of lens from the ectoderm of overlying the optic vesicle. Further lens induces the formation of cornea.

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