Lens fibre transdifferentiation in cultured larval *Xenopus laevis* outer cornea under the influence of neural retina-conditioned medium

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Received 25 June 1997; received after revision 2 September 1997; accepted 25 September 1997

**Abstract.** The outer cornea of larval *Xenopus laevis* can reprogram cell differentiation when cultured in medium conditioned by *X. laevis* neural retina (XRCM) or by *Rana esculenta* neural retina (RRCM). Under these experimental conditions corneal cells showed the same series of cytological changes of fibre cell differentiation observed during ontogenesis and in vivo lens regeneration: enlargement of nuclei and nucleoli, increase of ribosomal population (cytoplasm basophilia), cell elongation, gradual loss of basophilic properties and acquisition of acidophilic properties for crystallin synthesis and accumulation. These events were completely dependent on XRCM or RRCM, suggesting that the neural retina secretes a factor(s) which initiates and sustains lens fibre transdifferentiation of the corneal epithelial cells. This culture system appears to be a suitable one for investigating the control of lens fibre transdifferentiation in vitro.

**Key words.** Lens; transdifferentiation; *Xenopus.*

At present the available data concerning lens regeneration in Anura show that the phenomenon is restricted to larval *X. laevis* (for a review see [1, 2]). After lentectomy *X. laevis* larvae are able to regenerate a lens from outer cornea (squamous stratified epithelium two layers thick, bounded by a basement membrane) through a typical sequence of phases [3]. There is evidence that lens regeneration in this species occurs through a transdifferentiation process of corneal cells under the influence of a factor produced by the neural retina (for a review see [4]). Though the exact nature of the retinal factor has not yet been determined, some experimental results indicate that it has a protein nature; pellets of whole protein complement of the eye cup induce lens-forming transformation of the outer cornea [5] when implanted between the outer and inner cornea. The regenerative capacity, which is also present in the pericorneal epidermis, decreases during the larval period, and it disappears at metamorphosis when the outer cornea undergoes substantial structural changes [3]. Some experimental data suggest that the lens transdifferentiation process of the outer cornea requires a sequence of interactions extending over a long period of time during which the retinal factor must be present until complete lens transdifferentiation of cornea is achieved [6]. Although the lens transdifferentiative capacity of the outer cornea appears to be limited to larval *X. laevis*, the lens-inducing capacity of neural retina is widely present both in larval and adult Anura [2, 7, 8].

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The crucial role played by neural retina in the lens transdifferentiation of corneal cells has been confirmed by in vitro studies [9]. Lens-forming transformation of the outer cornea was completed in vitro when this tissue was cultured within the lentectomized eye cup. Fibre cells showing positive immunofluorescence with anti-total lens protein antibody were also observed when the outer cornea was cultured with isolated neural retina. In contrast, no lens fibre was formed when the outer cornea was cultured alone. Moreover, lens-forming structures originating from cornea at different times after lentectomy, regressed completely when isolated in vitro.

Lens transdifferentiation of the outer cornea in vivo and in vitro appears to occur without physical contact between this tissue and neural retina, suggesting that neural retina produces a diffusible factor needed to trigger reprogramming differentiation and maintenance of the lens-forming structure.

Recent experimental data [10] reveal that under in vitro conditions lens transdifferentiation of the outer cornea can be induced by bovine brain-derived acidic fibroblast growth factor (aFGF). These data together with the fact that the presence of FGFs and their receptors in ocular tissues of vertebrates has been evidenced in various studies (for a review see [11]), and that FGFs appear to be involved in the lens differentiation of mouse lens epithelial cells [12], suggest that one member of FGF family (for a review see [13–15]) could be the factor on which lens regeneration in larval *Xenopus* depends.

Because we are interested in determining the nature and mechanism of action of the putative inducing factor, we decided to establish a culture system with a powerful inducing capacity. In the present study we show that adult *X. laevis* and *R. esculenta* neural retina-conditioned medium (XRCM and RRCM) initiate fibre transdifferentiation in explant cultures of larval *Xenopus* outer cornea as determined by cell phenotype changes and initiation of crystallin synthesis. We used *X. laevis* anti-total lens protein antibodies to detect total lens crystallins and *R. esculenta* anti-γ-crystallin antibodies to detect γ-crystallin. The validity of using heterologous anti-γ-crystallin antibody against *X. laevis* lens was confirmed.

**Materials and methods**

**Animals.** Three experiments were carried out. In all experiments larvae of *X. laevis* at stage 51–52 (according to Nieuwkoop and Faber [16]) were used. All larvae were obtained from a single pair after amplexus and ovulation induced by gonadotropic hormones (Profasi, [10]).