In vitro lens transdifferentiation of *Xenopus laevis* outer cornea induced by Fibroblast Growth Factor (FGF)

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INTRODUCTION

Among Anura, lens regeneration appears to be an extremely limited phenomenon (reviewed by Reyer, 1977; Bosco, 1988a; Bosco et al., 1993a). Only *Xenopus laevis* larvae are capable of regenerating a lens after lentectomy (Freeman, 1963). This remarkable process results in transdifferentiation of the epithelial cells of the outer cornea (a two-layer thick squamous stratified epithelium, bounded by a basement membrane) which are able to reprogram differentiation into a new pathway for origination of the lens under the influence of the neural retina (reviewed by Bosco, 1988b). The regenerative capacity, which is also present in the pericorneal epidermis, decreases during the larval life and disappears at metamorphosis. The results obtained in grafting experiments have shown that lens regeneration in *Xenopus laevis* larvae can be stimulated by the neural retina isolated from larvae of different species which are not able to realize lens regeneration (Bosco et al., 1993b). Although during lens regeneration after lentectomy the stimulus for lens transdifferentiation of the outer cornea is provided by neural retina, there is evidence that, under different experimental conditions it can be supplied by other tissues, i.e., pituitary, spinal ganglia, limb bud, amputated hind limb, limb blastema and spinal cord (reviewed by Bosco, 1988b).

Recently, in vitro experiments have revealed that the outer cornea of larval *Xenopus laevis* undergoes lens transdifferentiation when isolated and cultured in the presence of bovine brain-derived acidic Fibroblast Growth Factor (aFGF) is able to reprogram the differentiation into lens fibers, although this transdifferentiative process is not coupled with the formation of a normally organized lens. The capacity of aFGF to promote lens differentiation from cornea is not linked to its mitogenic activity. The cultured corneal cells can transdifferentiate into lens fibers in the presence of aFGF when DNA replication and cell proliferation are prevented by addition of aphidicolin, a specific inhibitor of DNA polymerase in eukaryotes, to the culture medium.

Key words: lens, transdifferentiation, FGF, cornea, *Xenopus laevis*

MATERIALS AND METHODS

Animals

In all experiments larvae of *Xenopus laevis* at stage 51-52 (according to Nieuwkoop and Faber, 1956) were used. All larvae were obtained from a single pair after gonadotropic hormones induced amplexus and ovulation (Profasi, Serono). In all experiments, larvae were anaesthetized with MS 222 1:2000 before operation and fixation.

Explant culture

The larvae were immersed for 30 seconds in 1% Euclorine solution (Zambelletti), then rinsed three times in sterile Holtfreter’s solution. Operations were carried out in sterile Holtfrether’s solution containing 100 U/ml streptomycin and 0.25 µg/ml Fungizone (GIBCO). After a small incision in the dorsal pericorneal epidermis, the outer cornea was gently separated from the inner cornea with a thin hook-shaped tungsten needle, then, in order to isolate corneal fragments of standard size a Millipore filter disk, 1 mm in diameter, with a central hole, 0.20 mm in...
diameter, was placed under the outer cornea in such a way that the central hole was in front of the pupillary hole. The outer cornea and a small portion of pericorneal epidermis were removed through a circular incision along the edge of the filter disk. The tissues removed were rinsed four times in Leibovitz L 15 (Flow) diluted with water (2:1) and containing 100 U/ml penicillin, 100 μg/ml streptomycin (GIBCO), 1.5% glutamine (200 mM). The explanted tissues were placed in a plastic organ culture dish (35×10 mm Falcon Plastics) with 2 ml of Leibovitz L 15 medium diluted with water (2:1) and containing 100 U/ml penicillin, 100 μg/ml streptomycin, 1.5% glutamine (200 mM) and 10% inactivated foetal bovine serum (GIBCO). The culture medium was renewed daily.

**Histological methods**

Cultures and larvae were fixed in 95% ethanol at 4°C, embedded in paraffin, cut into 5 μm serial sections and stained using the Mallory-Azan’s method according to Heidenhain (1915).

**Cell number determination and mitotic index in corneal explants**

The statistical analysis was performed on five explant cultures at each fixation day.

Cell number determination was accomplished by counting all nuclei in all sections at 1000× magnification on a Leitz compound microscope, one eye-piece of which was fitted with a 1 cm² grid divided into 100 squares, following the method of Abercornzie (1946) for estimation of nuclear population from microtome sections. Mitotic index was calculated as a percentage of cells in mitosis of the total number of cells. Differences in mean between corneal explants of different experiment (Exp. I,II,III,IV) were analysed for significance with ANOVA followed by TUKEY post-test.

**Preparation of lens-specific antibodies and retrospective immunofluorescence staining**

*Xenopus laevis* adults were killed, lenses were removed and freed from *Xenopus laevis* lens fibres (early stage 4 of Freeman, 1963) as previously shown by Brahma and McDevitt (1974).

Four types of experiments were carried out:

**Experiment I. Simple lentectomy** (30 cases). This experiment was carried out to determine the percentage of larval *Xenopus laevis* showing lens regeneration, as this percentage may vary considerably (Waggoner and Reyer, 1975). The lenses were removed by the operative technique described in a previous paper (Bosco, 1988a). Operations were performed in Holtfreter’s solution and subsequently the larvae were maintained in Holtfreter’s solution for 3 days before being transferred to spring water. Entire animals were fixed 3, 5, 7, 10 and 15 days after operation.

**Experiment II. Explant culture of outer cornea and pericorneal epidermis** (control experiment, 140 cases). The cultures were ended after 2, 3, 4, 5, 6, 7 and 10 days.

**Experiment III. Explant culture of outer cornea and pericorneal epidermis with aFGF** (140 cases). The medium used in these cultures was supplemented with 500 ng/ml of bovine brain-derived acidic Fibroblast Growth Factor (Sigma N° F5267). The cultures were ended after 2, 3, 4, 5, 6, 7 and 10 days.

**Experiment IV. Explant culture of outer cornea and pericorneal epidermis with aFGF and aphidicolin** (140 cases). This experiment was carried out with the aim of establishing whether the lens transdifferentiation of aFGF-stimulated corneal cells was related to nuclear DNA replication. Aphidicolin, a mycotoxin, is a specific inhibitor of nuclear DNA replication in eucaryotes, acting as an inhibitor of DNA polymerase α activity (Spadari et al., 1982). Initially, the action of aphidicolin was tested on explant cultures of outer cornea (100 cases). The medium was supplemented with 10 μg/ml of aphidicolin (Sigma N° C-6255). Each culture dish, containing 10 explants in 2 ml of medium, was supplemented with 0.2 ml of colchicin (10⁻⁴ M in Holtfreter’s solution) 12 hours before being fixed (Freed and Freed, 1970). The cultures were ended after 1, 2, 3, 4 and 5 days. The histological examination of the 5-μm thick serial sections showed that no mitotic figure was present after 2 days in culture. Subsequently, the explanted outer cornea was cultured in a medium containing 10 μg/ml of aphidicolin (Ikegami et al., 1978) for 2 days, when the medium containing the inhibitor was supplemented with 500 ng/ml of aFGF. The cultures were...
ended at 2, 3, 4, 5, 6, 7 and 10 days after the growth factor addition. Each culture dish was supplemented with 0.2 ml of colchicin (10^{-4} M) 12 hours before being fixed.

In all culture experiments, 10 explanted tissues were fixed at the time of explantation and used as explant controls (day 0).

RESULTS

Experiment I. Simple lentectomy

In 26 out of the 30 cases examined (Table 1) the outer cornea underwent lens-forming transformation. The results on the regenerative process were consistent with those reported by Freeman (1963).

Experiment II. Explant culture of outer cornea and pericorneal epidermis

Results refer to cytological analysis performed on explants fixed after 4 and 10 days, these explants being the most representative of the differentiative events.

None of the 100 cases examined after 4 (50 cases) and 10 (50 cases) days showed corneal and/or pericorneal lens transformation (Table 1). In most cases (72), the cultured tissues originated large hollow vesicles (Fig. 1A); anyway, both the outer cornea and pericorneal epidermis maintained the same epithelial tissue organization as that of a normal eye. In 9 cases the volume of the double epithelial sheet was observed to be slightly increased (Fig. 2B), as was sporadically (11 cases) the number of the melanocytes originally present only in the pericorneal epidermis. These results fully confirmed those we had previously obtained (Bosco et al., 1993b). The immunofluorescence test gave negative results in all 50 cultures examined (Fig. 2).

Table 1. Summary of the results of experiments to determine lens transdifferentiation under different in vitro conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>S.L. Nº</th>
<th>E.C. Nº</th>
<th>E.C. + aFGF Nº</th>
<th>E.C. + Aph + aFGF Nº</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nº of cases</td>
<td>30</td>
<td>140</td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td>Nº of lens</td>
<td>26</td>
<td>0</td>
<td>86</td>
<td>64</td>
</tr>
<tr>
<td>transdifferentiation</td>
<td>87</td>
<td>0</td>
<td>61</td>
<td>46</td>
</tr>
</tbody>
</table>

S.L., simple lentectomy (Exp. I).
E.C., explant culture of outer cornea (Exp. II).
E.C. + aFGF, explant culture of outer cornea + aFGF (Exp. III).
E.C. + Aph + aFGF, explant culture of outer cornea + Aphidicolin + aFGF (Exp. IV).

Experiment III. Explant culture of outer cornea and pericorneal epidermis with aFGF

Histological examination showed that after 3 days the proliferation (Table 2; Fig. 3) of the explanted tissues resulted in a progressive filling (Fig. 4A) of the hollow vesicles that were observed after 1 and 2 days. This process gradually gave rise to solid cell aggregates (Fig. 4B,C). Typical cytological signs of lens differentiation (Eguchi and Okada, 1973; Freeman, 1963; Papaconstantinou, 1967; Yamada et al., 1973), such as cell...
elongation, granular cytoplasm, and increasing cytoplasmic acidophilia were clearly observed after 4 days in culture (Fig. 4B,C). Lens transdifferentiation was observed in 86 out of 100 explant cultures (Table 1) ended after 4, 5, 6, 7 and 10 days. In most cases, the whole cultured tissue underwent lens-forming transformation giving rise to lens differentiating structures which did not show the normal fibers arrangement of the lens: lens fibers being oriented in the anterior-posterior plane rather than concentrically. Only in 3 cases, inside the lens forming structures, were there normally arranged primary and secondary lens fibers coexisting with different amounts of irregularly arranged fibers (Fig. 4C,D). After 10 days, the degree of differentiation of the explanted tissues was higher (Table 2). This became apparent as a gradual disappearance of lens fiber cellular nuclei and lens fibers aggregating into the so-called ‘primary lens fiber nucleus’ as it occurs during lens development and regeneration in vivo (Freeman, 1963). In most cases thick ‘nuclei’ of lens fibers were scattered among less thickened lens fibers (Fig. 4A). Sometimes the whole cultured tissue gave rise to a very thickened lens fibers aggregate similar to a large primary nucleus (Fig. 4B).

The immunofluorescence method with absorbed anti-total lens proteins showed the specificity of the lens-forming structures and confirmed the histological observations. No reaction occurred in the 2 and 3 day explant cultures. The majority of 4 day explant cultures showed a positive reaction in a small number of cells in the internal part of the lens-forming structure (Fig. 5B). Subsequently, the explant cultures showed positive cells which progressively increased in number and intensity (Fig. 5C,D). The cultured tissues were entirely positive after 10 days (Fig. 5E,F).

**Experiment IV. Explant culture of outer cornea and pericorneal epidermis with aFGF and aphidicolin**

The cultured tissues neither proliferated (Table 3) nor produced hollow vesicles, however, the corneal cells clustered, giving rise to small solid aggregates whose volume remained almost unchanged throughout the experiment, except for a slight increase due to cell elongation. Under these experimental conditions the typical cytological changes of lens-differentiation were observed starting from days 5 of culture. Lens transdifferentiation was observed in 64 out of 80 (80%) explant cultures ended after 5, 6, 7 and 10 days. The immunofluorescence test confirmed the

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**Table 2. Experiment III. Explant culture of outer cornea and pericorneal epidermis with aFGF (E.C. + aFGF). Lens transdifferentiation with reference to days in culture**

<table>
<thead>
<tr>
<th>Days in culture</th>
<th>Nº of cases</th>
<th>Lens fiber formation %</th>
<th>Primary nucleus-like structures %</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>15</td>
<td>75</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>6</td>
<td>20</td>
<td>19</td>
<td>95</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>17</td>
<td>85</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>19</td>
<td>95</td>
</tr>
</tbody>
</table>

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**Fig. 4.** Explant culture of outer cornea and pericorneal epidermis with aFGF. (A) 3-day culture: proliferation of the explanted tissues produced a progressive filling of the hollow vesicles observed after 2 days. (B) Typical cytological signs of lens differentiation could be clearly observed after 4 days in culture. (C,D) 7-day culture: two lens-forming structures containing different amount of lens fibers regularly (arrows) and irregularly arranged (arrowheads). Note the different ratio of regular versus irregular areas in the two structures. Bars 10 μm.
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...showed that the lens-transdifferentiative process was in progress (Fig. 6A-D).

**DISCUSSION**

**Lens transdifferentiation of cornea stimulated by aFGF**

The above results indicate that the outer cornea of larval *Xenopus laevis* can reprogram cell differentiation into lens fibers when cultured in the presence of brain-derived aFGF. Under the present experimental conditions, corneal cells show the same series of cytological changes of fiber cell differentiation as those observed during ontogenesis (Papaconstantinou, 1967) and in vivo lens regeneration (Freeman, 1963): 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 (McDevitt and Brahma, 1973; Brahma and McDevitt, 1974). The present experiment, however, did not examine whether the expression of crystallin genes follows the same pathways as previously determined during lens development and regeneration in vivo (McDevitt and Brahma, 1973; Brahma and McDevitt, 1974). This requires further investigation with specific antisera directed against each antigen class (α, β and γ crystallins). However, the lens-forming structures, in general, were not organised into normal lens, since their lens fibers arrangement was irregular and a lens epithelium was not evident. During in vivo lens regeneration, the lens fiber differentiation follows a set pattern. The first few lens fibers are irregularly arranged in the forming lens, and are joined by other cells which, in turn, produce lens fibers, to form the primary lens fiber nucleus; subsequently, secondary lens fibers are formed and start to grow from the equatorial zone of a lens epithelium and the primary lens fiber nucleus gradually disappears. The result of this morphogenetic process is a regenerated lens showing the same structure as a normal lens. The same result is sometimes obtained in explant culture of a lentectomized eye cup with the outer cornea implanted into the vitreous chamber (Bosco et al., 1993c). Evidently, the normal lens structure can be realised under biological and/or physical conditions (by supposition: gradients of morphogenetic factors, intracellular pressure) which were not present in our experiments.

The fact that aFGF can promote lens-transdifferentiation of corneal cells suggests that this or another member of the FGF family (Baird et al., 1986; Burgess and Maciag, 1989) may be the factor produced by neural retina which the lens regeneration depends on. FGF was originally isolated from bovine brain and pituitary (Gospodarowicz et al., 1975). Subsequently, several growth factors of peptide nature, all potent mitogens for mesenchymatous cells have been discovered in a variety of tissues including bovine cartilage, hypothalamus, retina, macrophages, corpus luteum, adrenal glands, rat chondrosarcoma and human brain. Physical-chemical analyses of these mitogens by SDS/Page, isoelectric focusing and in particular the assessment of their respective binding affinities for heparin have shown that

**Table 3. Cell numbers, number of mitoses and mitotic index in control explant cultures and in explant cultures treated with aFGF or with aFGF plus Aphidicolin**

<table>
<thead>
<tr>
<th>Days</th>
<th>Total number of cells</th>
<th>Total number of mitosis</th>
<th>Mitotic index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E.C.</td>
<td>E.C. + aFGF</td>
<td>E.C. + Aph + aFGF</td>
</tr>
<tr>
<td>0</td>
<td>2684±398</td>
<td>3100±247</td>
<td>2876±275</td>
</tr>
<tr>
<td>2</td>
<td>2713±204</td>
<td>7848±551*</td>
<td>2715±306</td>
</tr>
<tr>
<td>3</td>
<td>2640±167</td>
<td>13223±424*</td>
<td>2647±298</td>
</tr>
<tr>
<td>4</td>
<td>2782±266</td>
<td>13942±687*</td>
<td>2715±234</td>
</tr>
<tr>
<td>5</td>
<td>2900±250</td>
<td>12160±603*</td>
<td>2648±236</td>
</tr>
<tr>
<td>7</td>
<td>3206±355</td>
<td>10146±272*</td>
<td>2506±258</td>
</tr>
</tbody>
</table>

E.C., control explant culture (Experiment II)
E.C. + aFGF, explant culture treated with aFGF (Experiment III)
E.C. + aFGF + Aph, explant culture treated with aFGF plus Aphidicolin (Experiment IV)

Aph, Aphidicolin

Values expressed as means ± s.d.

*Statistically significant difference (P<0.001) with respect to the same group at day.
†Different with respect to E.C. at the same day

Treatment with aphidicolin was started 2 days before addition of a FGF (day 0).

**Fig. 5. Explant culture of outer cornea and pericorneal epidermis with aFGF.**

(A) 10-day culture: thick lens fiber nuclei were scattered among less thickened lens fibers. (B) Sometimes the whole cultured tissue gave rise to lens fiber aggregates similar to a large lens fiber primary nucleus. Bars 10 μm.
two classes of identical or very closely related growth factors exist. Class I heparin-binding growth factors (HBGFs), with an acidic pI (4.8-6) include acidic fibroblast growth factor (aFGF), endothelial cell growth factor (ECGF), eye-derived growth factor II (EDGF-II), c-retina-derived growth factor (cRDGF), anionic hypothalamus-derived growth factor (HDGF), brain-derived (BDGF) and astroglial growth factor I (AGF-I).

Class 2 HBGFs with a basic pI (8-10) include basic FGF (bFGF), tumour angiogenesis factor (TAF), eye-derived growth factor I (EDGF-I), β retina-derived growth factor (βRDGF), cationic hypothalamus-derived growth factor (CHDGF), macrophage-derived growth factor (MDGF), astroglial growth factor (AGF) and cartilage-derived growth factor (CDGF), (for a review see Deuel, 1987; Gospodarowicz, 1990; Gospodarowicz et al., 1987; Lobb et al., 1986).

Moreover, the two prototype members of the FGF family, aFGF and bFGF, show a significant structural homology (Esch et al., 1985; Gospodarowicz, 1990). The range of cellular types capable of producing FGFs and of responding to their action is becoming ever larger (Mercola and Styles, 1988; Sporn and Roberts, 1988, 1990). Increasing evidence shows that those peptides play an important role during morphogenesis (Gospodarowicz, 1990; Hollenberg, 1989; Lee and Han, 1990; Mercola and Stiles, 1988; Slack and Isaacs, 1989; Slack et al., 1987; Song and and Slack, 1994; Whitman and Melton, 1992). The presence of FGFs and their receptors in ocular tissues of vertebrates has been shown by several different studies (for a review see Park and Hollenberg, 1993), and different actions on ocular tissues have been suggested. The FGFs appear to stimulate the division of lens and corneal epithelial cells (Gospodarowicz et al., 1977a), scleral fibroblasts (Del Vecchio et al., 1988), corneal and capillary cells (Bensaid et al., 1989; Gospodarowicz et al., 1977b) and adult bovine Muller cells (Mascarelli et al., 1991). A regulating role of FGFs in lens fiber differentiation has also been proposed. It has been shown that FGFs are able to stimulate cultured mouse lens epithelial cells to proliferate, migrate and differentiate into lens fibers in a progressive concentration-dependent manner (McAvoy and Chamberlain, 1989). On the basis of experimental data, Schulz et al. (1993) proposed that the distinct anterior-posterior pattern of lens differentiation in mouse in vivo could be explained in terms of bio-availability of bFGF and aFGF and their molecular forms in the ocular media.

Recent experiments (Park and Hollenberg, 1989, 1991) have shown that in the chick embryo the state of differentiation of retinal pigmented epithelium can be altered in vivo by specific growth factors, i.e. aFGF and bFGF.

Among the various tissues tested for their ability to stimulate lens regeneration in the outer cornea of Xenopus laevis, neural retina, spinal ganglia, pituitary and limb bud are particularly effective (Bosco, 1988b; Reeve and Wild, 1981). Note that brain, retina and pituitary are sources of FGFs (Baird and Böhlen, 1990; Gospodarowicz, 1990; Gospodarowicz et al., 1987), and that FGF gene expression has been shown in mouse limb bud (Beverly and Goldfarb, 1993).

The above reported data together suggest the hypothesis that a member of the FGF family may be the factor that the in vivo lens regeneration from cornea depends on.

In the in vitro study of the capacities of different eye tissues to differentiate lens and the identification of a factor as a natural promoter of lens fiber differentiation are complicated because lens fiber differentiation may be influenced by different agents present in serum added to the culture medium (Piattigorsky, 1981). In this connection, the in vitro corneal lens transdifferentiation of Xenopus seems to be peculiar in that isolated outer cornea cultured in a 10% foetal bovine serum supplemented medium does not appear to be able to achieve lens transdifferentiation (Bosco et al., 1993c). Indeed the presence of FGF in bovine, human and rat serum was shown by radioimmunoassay (reviewed by Baird et al., 1993).

Fig. 6. Immunofluorescence analysis of anti-total-lens proteins in explant culture of outer cornea and pericorneal epidermis with aFGF. (A) No reaction occurred in 2- and 3-day cultures. (B) 4-day culture: the explant culture showed a positive reaction in a small number of cells in the internal part of the lens-forming structures. (C) 5-day culture; (D) 6-day culture: the cultured tissues showed positive cells which progressively increased in number and intensity. (E,D) 10-day culture: the cultured tissues were entirely positive. Bars 10 μm.
1986), but in each of these species ir-FGF circulated as a 150×10^3 M_r molecule, which is quite different from the biologically active 16×10^3 M_r FGF. It has been suggested that the serum form of FGF needs enzymatic cleavage to become biologically active (Baird et al., 1986), but this process cannot occur in corneal tissue culture. Other hypotheses can be made to explain the failure of serum FGF to promote lens transdifferentiation. FGF may act in a concentration-dependent manner, and the amount of this factor in the culture medium may not be suitable to trigger lens transdifferentiation. Last but not least, the heat treatment for inactivation (56°C, 40 minutes) may deprive the FGF of its lens transdifferentiation promoting activity.

Alternatively, we cannot discard the possibility that the aFGF may inactivate some serum factor which inhibits transdifferentiation.

**Lens transdifferentiation of cornea without preceding cell division**

The results obtained under the present experimental conditions show that in vitro corneal cells can transdifferentiate into lens fibers in the presence of aFGF when DNA replication and cell proliferation are prevented by the presence of aphidicolin, a specific inhibitor of DNA polymerase in eukaryotes (Spadari et al., 1982). These data are consistent with those recently obtained in vivo (Filoni et al., 1995) by implant experiments where enough inhibition of corneal cell proliferation was obtained by treatment with mitomycin C, an antibiotic able to bind DNA and interfere with its function (Kazumitsu and Komano, 1984; Rodighiero et al., 1978; Warning, 1968).

The relationship between cell division and cell differentiation is one of the most controversial questions in developmental and cell biology (for a review see Okada, 1991). The interdependence of these two cellular processes has been expressed as the quantal cell cycle theory (Holtzer et al., 1972). At present, the available data do not settle this question. In some systems, such as gene expression in early developmental stages of *Xenopus* (Newport and Kirschner, 1984), transdetermination of *Drosophila* imaginal discs (Gehring, 1978), newt lens regeneration from dorsal iris pigmented epithelium (Yamada, 1982, 1989; Yamada and McDevitt, 1984; Yamada et al., 1975), transdifferentiation of neural retina into pigmented cells (Pritchard et al., 1978), and lentoid formation from clonal outgrowth of a single culture of retinal pigmented epithelium (Eguchi, 1979), a fixed number of DNA replications appear to be a prerequisite for specific gene expression. In other systems, such as, transformation of secretory cells into ectodermal muscle cells in *Hydra* (Burnet, 1968); transdifferentiation of cross-striated muscle cells in hydromedusae *Podocoryne carneae* (Schmid, 1975), and conversion of Triturus xantochroites into melanophores (Frost et al., 1984), transdifferentiation seems to occur without any prior DNA replication (for a review see Bedsford, 1990). Lens transdifferentiation of *Xenopus* corneal cells appears to be included in the last of the above reported categories. There is evidence that during lens regeneration from cornea in larval *Xenopus laevis*, DNA synthesis begins in the corneal cells as early as 2 days after lentectomy and continues throughout the regeneration period until the inner cells differentiate into lens fibers just before the production of crystallins; afterwards this process ceases in the lens fibers and becomes restricted to lens epithelium (Brahma and McDevitt, 1974; Waggoner and Reyer, 1975). The lens transdifferentiation of corneal cells into lens fibers occurring in corneal tissue culture in the presence of FGF and aphidicolin has never been associated with real lens formation, hence DNA synthesis, cell proliferation and its persistence in the lens epithelium may be necessary for regeneration of a true lens and its maintenance, but not be so necessary for the lens transdifferentiation step.

**REFERENCES**


Fig. 7. Explant culture of outer cornea and pericorneal epidermis with aFGF and aphidicolin. (A-D) The immunofluorescence test with anti-total-lens proteins showed the lens specificity of the cultured tissues and the progressive lens transdifferentiation. (A) 4-day culture; (B) 7-day culture (C,D) 10-day culture. Bars 10 μm.


