Fibroblast growth factor (FGF) induces different responses in lens epithelial cells depending on its concentration

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Summary

We reported previously that epithelial cells in explants from neonatal rat lenses, when cultured in the presence of fibroblast growth factor (FGF), showed increased proliferation, cell migration and fibre differentiation; moreover, fibre differentiation in response to the basic form of FGF (bFGF) was virtually completely blocked by an anti-bFGF antibody. In the present study, we report a detailed analysis of the effects of bFGF on cells in the central region of lens epithelial explants. Proliferation in explants was assessed by measuring $[^{3}H]$thymidine incorporation. Cell migration was measured by labelling cells in explants with fluorescein isothiocyanate (FITC)-dextran and monitoring them by UV fluorescence microscopy. Fibre differentiation in explants was assessed on the basis of $\beta$-crystallin accumulation. This study showed that half maximal activities for the three responses, proliferation, migration and fibre differentiation, were achieved at different concentrations of bFGF, namely, 0.15, 3 and 40 ng ml$^{-1}$, respectively. Thus, the response of lens epithelial cells to bFGF varied qualitatively, as well as quantitatively, as the concentration increased. Monitoring FITC–dextran injected cells for up to 5 days after exposure to bFGF allowed analysis of the interrelation between various responses to bFGF in individual cells. As expected some cells divided in response to FGF, mainly within the first three days. However, whether or not they divided, all labelled cells responded to FGF by migrating and elongating. Maximal migration occurred during the first day of culture and maximal elongation was achieved by day 4. It was also found that $[^{3}H]$thymidine incorporation and cell migration were substantially inhibited (90–93 %) by the anti-bFGF antibody shown previously to inhibit $\beta$-crystallin accumulation, providing strong support for the conclusion that FGF is the molecule responsible for inducing all three responses. All these findings lead us to suggest that variations in FGF concentration in the different ocular media may be involved in controlling and maintaining lens polarity and lens growth patterns.

Key words: lens, lens induction, lens fibre differentiation, fibroblast growth factor, mitosis, migration, crystallins.

Introduction

The eye lens is made up of two forms of cells contained within a thickened basal lamina known as the lens capsule (Fig. 1). Long transparent fibre cells make up the bulk of the lens, while a monolayer of epithelial cells covers the anterior surface of the fibres. In mammals, the epithelial cells contain $\alpha$-crystallin and the fibre cells contain $\beta$- and $\gamma$- as well as $\alpha$-crystallins (McAvoy, 1980a; Piatigorsky, 1981; Yancey et al. 1988). Cell division is restricted to the epithelium. Most divisions occur in a region just above the lens equator known as the germinative zone (Harding et al. 1971; McAvoy, 1978; Fig. 1). Progeny of epithelial cell divisions elongate at the equator and synthesize $\beta$- then $\gamma$-crystallins as they differentiate into fibre cells (McAvoy, 1978). By this process, new fibre cells are continuously added to the fibre mass at all ages (Messier and Leblond, 1960; Hanna and O'Brien, 1961; Brolin et al. 1961). Thus, the lens continually grows throughout life and maintains its distinct polarity with the monolayer of epithelial cells covering only the anterior half of the fibres.

There is evidence that lens polarity is determined by the spatial relationship of the lens to the neural retina. In the normal lens, epithelial cells are only found on the surface of the lens that faces away from the neural retina (Fig. 1). However, in lens inversion experiments, Coulombre and Coulombre (1963) turned the chicken lens through 180° so that the epithelial cells that normally faced the cornea then faced the neural retina. In this new environment, the epithelial cells elongated and gave rise to fibre cells. Lens inversion experiments with mouse eyes gave similar results (Yamamoto, 1976). In the latter experiments, it was also shown that the elongation of epithelial cells into fibres depended on the continued presence of the neural retina.

To investigate the nature of the lens fibre-inducing influence from the neural retina, we developed an explant culture system. Epithelial explants from newborn rat lenses were grown either in unsupplemented medium or in combination with neural retinas or retina-
cells and fibre cells occurs in the vicinity of the lens zone (indicated by asterisks) which are bathed by the aqueous of the posterior chamber (PC). For further details, see text.

Fig. 1. Diagram showing the anatomy of the eye. The two forms of lens cells are contained within a thickened basal lamina, the capsule (Ca). The transition between epithelial cells and fibre cells occurs in the vicinity of the lens equator. The central epithelial cells are bathed by the aqueous of the anterior chamber (AC). Proliferation in the lens occurs largely in the epithelial cells of the germinative zone (indicated by asterisks) which are bathed by the aqueous of the posterior chamber (PC). For further details, see text.

conditioned medium (RCM). In the presence of neural retinas or RCM, cells in explants elongated and synthesized β- and γ-crystallins, undergoing morphological and molecular events characteristic of fibre differentiation in the intact lens (McAvoy, 1980b; Campbell and McAvoy, 1984; Walton and McAvoy, 1984). Neural retinas and RCM were also shown to induce cell proliferation in explants (McAvoy and Fernon, 1984) and RCM was shown to stimulate cell migration (McAvoy, 1988).

Recently, we identified acidic and basic fibroblast growth factors (aFGF and bFGF) purified from neural retina or brain as potent fibre cell-inducing molecules; both induce the accumulation of β-crystallin in epithelial explants accompanied by cell elongation and other characteristic morphological changes observable by light microscopy (Chamberlain and McAvoy, 1987, 1989). Moreover, in immunoneutralization studies, we showed that these responses to bFGF were almost completely blocked by an anti-bFGF antibody (Chamberlain and McAvoy, 1989). Preliminary experiments showed that FGF also stimulated cell migration and cell proliferation in lens explant cultures (Chamberlain and McAvoy, 1989).

We now report a detailed analysis of the effects of bFGF on cells in the central region of the lens epithelium. First, using epithelial explants, we determined the concentration dependence of the three responses to bFGF, proliferation, cell migration and fibre differentiation. Proliferation was assessed by determining [3H]thymidine incorporation. Cell migration was measured by labelling single cells with FITC–dextran and monitoring them by UV fluorescence microscopy; this also allowed the proliferation and cell elongation responses of individual cells to be analysed. Fibre differentiation was assessed by measuring the accumulation of β-crystallin. We also investigated the effect of the anti-bFGF antibody on cell migration and proliferation under these culture conditions. The possible significance of these findings to our understanding of lens differentiation is discussed.

Materials and methods

The FGF used throughout this study was bFGF of high purity, as assessed by SDS–polyacrylamide gel electrophoresis, prepared from bovine brain and purified on Sepharose chromatography as described in detail elsewhere (Chamberlain and McAvoy, 1989). FGF was lyophilized in the presence of bovine serum albumin, stored at —20 °C and used within 4 months of preparation. Under these conditions FGF retained full activity, as assessed by its ability to induce β-crystallin accumulation in lens epithelial explants. Experiments were designed so that the various activities of FGF were assayed in parallel to ensure valid comparison.

Explants of lens epithelial cells were prepared from 3-day-old Wistar rats, pinned out on their capsule in serum-free medium 199 containing bovine serum albumin and cultured as described previously (Chamberlain and McAvoy, 1989).RIA grade bovine serum albumin (Sigma, St Louis, MO, USA, Cat No. A7888) was used throughout this investigation. Explants to be used for the assessment of β-crystallin accumulation or [3H]thymidine incorporation were trimmed to remove the peripheral region containing the germinative zone (McAvoy and Fernon, 1984; Fig. 1). Untrimmed explants were used for the FITC–dextran cell labelling studies. Routinely, fresh M199 (for control explants) or M199 containing bFGF (for FGF-treated explants) was added on the day of preparation of the explants, day 0, and explants were cultured for up to 5 days without replacing the medium. For FITC–dextran cell labeling studies, each culture dish contained only one explant; otherwise, dishes contained two explants.

Explants were lysed in 0.02% Triton X-100 – 10 mM EDTA, pH 12-3 (Richardson and McAvoy, 1986) and β-crystallin in the lysates was determined by an ELISA (Richardson and McAvoy, 1986). Cell proliferation was assessed by measuring the incorporation of [3H]thymidine (Chamberlain and McAvoy, 1989), the incorporation period being 18–24 h unless otherwise specified. Immunoneutralization studies were carried out as described previously using IgG derived from antisera raised against a synthetic peptide corresponding to the first 24 aa of bovine β-crystallin (Chamberlain and McAvoy, 1989); this antibody is specific for bFGF (A. Baird, personal communication). In these experiments, anti-bFGF IgG (or non-immune IgG) was included throughout the culture period.

For the FITC–dextran cell labelling studies, the central region of each explant was divided into nine squares and three cells per square, separated by at least 60 μm, were injected with FITC–dextran (McAvoy, 1988). Cells were visualized using an inverted microscope (Diavert, Wild-Leitz) equipped for fluorescence microscopy and monitored at 24 hourly intervals as described previously (McAvoy, 1988) with the following modifications. During monitoring of cells, a neutral density filter (N16, Wild-Leitz) which reduced UV irradiation by about 90% was inserted into the microscope system. To
Responses of lens cells to FGF

allow visualization of the cells under these conditions, a more sensitive camera (National Panasonic Low Lite WV-1900) was substituted for the camera used in the previous study.

For assessing changes in cell morphology, the following definitions were used: length was determined by joining the two most widely separated points on the cell perimeter with a line that followed the contour of the cell; width was determined at the midpoint of the line representing length and in the dimension perpendicular to it.

Data were analyzed by standard statistical methods (Snedecor and Cochran, 1967). For testing the significance of differences, Student's t-test was applied where the distribution of data was approximately normal and sample variances were comparable according to the F test; otherwise Wilcoxon's ranking test was used.

Results

Dose response characteristics for cell proliferation, cell migration and fibre differentiation

Experiments were carried out using a range of doses of bFGF (Fig. 2). As the concentration of FGF was increased, the cells first began to proliferate as shown by increased incorporation of [³H]thymidine into explants. A higher concentration of FGF was required to stimulate cell migration as assessed by recording movements of FITC-labelled cells. Fibre differentiation as assessed by β-crystallin accumulation required an even higher concentration of FGF. The half maximal doses for these three responses were 150 pg ml⁻¹, 3 ng ml⁻¹ and 40 ng ml⁻¹, respectively. The three responses were not mutually exclusive, although each appeared to be at least partially inhibited at concentrations of FGF greater than the optimal for that response.

Migration and cell proliferation were assessed within the first 24 h of the addition of FGF, whereas β-crystallin was determined in explants cultured for a further 4 days without replacing the medium. Therefore, the apparent requirement for a higher dose of FGF for β-crystallin accumulation may have arisen from depletion of FGF during the culture period. To test this possibility, we carried out additional experiments in which the medium was replaced with fresh medium containing bFGF at the original concentration on each of the first three days of culture. Replenishing the supply of FGF did not significantly alter the dose required for half maximal stimulation of β-crystallin accumulation when compared with the routine procedure (data not shown).

Since migration can only be measured in epithelial cells in the central region of explants, the other responses reported in Fig. 2 (proliferation and β-crystallin accumulation) were also measured in the central region only, using explants trimmed to remove the peripheral region. However, FGF also induces cells in the peripheral region to accumulate β-crystallin (Chamberlain and McAvoy, 1989) and proliferate (data not shown). The results of preliminary experiments indicate that peripheral cells are more responsive to bFGF than central cells, at least with respect to the accumulation of β-crystallin. For example, cells in the peripheral region (that is, in explants trimmed to remove the central region) accumulated 3.9 ± 0.5 μg explant⁻¹ (mean ± s.e.m., 7 dishes) in response to 6 ng ml⁻¹ bFGF, a dose that was not sufficient to induce significant accumulation of β-crystallin in central cells (Fig. 2C).

FITC-dextran cell labelling studies

To quantify cell migration and to analyze the inter-
relation between the responses of individual cells to FGF, single cells in lens epithelial explants were injected with FITC-dextran and observed by UV fluorescence microscopy. The epithelial explants are composed of a monolayer of tightly packed epithelial cells attached to the lens capsule. Labelled cells within this monolayer can be clearly visualised (Fig. 3). Two separate experiments were carried out; each included both control and FGF-treated explants and about 30–50 cells per treatment group, injected on day 0. As preliminary analysis showed that both experiments gave essentially the same results, the data from the two experiments were pooled. Overall, in control and FGF-treated explants, respectively, about 77 % and 97 % of cells survived the first 24 h period following injection and, of these cells, 45 % and 85 % survived and could be clearly visualized throughout the 5 day culture period. This represents a significant improvement in survival compared with a previous study in which cells were subjected to more intense UV irradiation during fluorescence microscopy (McAvoy, 1988).

Cell proliferation
When FITC-dextran-labelled cells divided during the culture period, each daughter cell retained sufficient label to be visualized. The survival rates were comparable in cells that divided and those that did not. As would be expected from the results of the [3H]thymidine incorporation experiments described above (Fig. 2A), bFGF at the concentration used in the cell labelling study, 125 ng ml⁻¹, significantly stimulated the rate of cell division in lens epithelial explants. The number of cells that divided increased from 3 % in control explants to 24 % in FGF-treated explants (P < 0.01) and most cell divisions occurred early in the culture period (Table 1).

Cell migration
Sequential recordings of the positions of labelled cells at 24 hourly intervals over a 5 day period showed that they moved laterally within the explant both in the presence and absence of FGF (Fig. 4). The direction of movement within the explant appeared to be random; individual cells often changed direction and no characteristic movement patterns were observed over the 5 day period, as illustrated for FGF-treated explants in Fig. 5. In control explants, the daily distances covered by cells were variable; some cells showed no movement whereas others moved up to 48 μm. The mean daily distance covered did not vary significantly throughout the culture period and, on average, the cells moved about 17 μm per day (Fig. 4).

For FGF-treated explants, there was a significant stimulation of migratory activity during the first 24 h after addition of FGF (Fig. 4). Cells developed lamellae and frequently became attenuated (Fig. 3B), changes which are characteristic of actively migrating cells (Trinkaus, 1984). When cells divided in response to FGF, their migratory behaviour was comparable in each of the progeny and was not significantly different from that of cells that did not divide; pooled data are shown in Fig. 4. The distances covered by individual cells were highly variable, ranging from 0 to 180 μm, but on average the cells moved about 2–8 times further than cells in control explants during this period. Between days 1 and 2, migration decreased but was still significantly higher than for cells in control explants. Subsequently, the mean daily distance travelled diminished to values comparable to or even less than the corresponding control values.

Note that, in this study, the lateral movement of an individual cell during each 24 h interval between measurements was represented by a straight line (see Fig. 5) and the length of this line was used for quantifying cell migration. Consequently, the distance travelled by any cell that changed direction during this period would have been underestimated. It is therefore likely that mean distances travelled were even greater than shown in Fig. 4, while the variance of measurements is probably less than indicated.

In both control and FGF-treated explants, migratory behaviour was not dependent on the position of the cell within the explant (central square 5 compared with remaining peripheral squares, see Fig. 5) or on the position of the cell relative to the nearest cut edge of the grid square at the time of injection.

Cell elongation
In control explants, there were no major changes in cell morphology throughout the culture period (Fig. 6; Table 2), but there was a small but significant increase in cell length as the culture period progressed (Fig. 7), especially in the outer grid squares of the explants. This was probably the result of a gradual loss of some epithelial cells from the monolayer (cf. McAvoy and

Fig. 3. Fluorescence micrographs of FITC-dextran labelled cells cultured for 24 h in control medium (A) or in medium containing bFGF, 125 ng ml⁻¹ (B). The bar represents 10 μm.
Table 1. Cell proliferation in control and bFGF-treated explants

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>FGF-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Number of injected cells surviving at the end of day 1; if division occurred during this period, each pair of daughter cells was counted as one original cell.

Fig. 4. Daily distances migrated by labelled cells over a 5 day period in explants cultured in control medium (broken line) or medium containing bFGF (solid line). Each value represents the mean ± s.e.m. of 28–29 or 70–78 individual cell measurements for control and FGF-treated explants, respectively. Data were derived from all cells, divided and non-divided, that survived the 5 day culture period. The asterisk indicates that this value is significantly different from the corresponding control value, P < 0.002.

Fig. 5. Patterns of cell migration in a typical explant cultured in medium containing bFGF, 125 ng ml⁻¹, for 5 days. One cell in each of six of the marked squares survived the FITC-dextran injection and could be visualized by fluorescence microscopy. Cell positions within their respective squares were marked at daily intervals (●) commencing on day 0 (○). In square 1, the labelled cell divided within the first day, while in squares 8 and 6 division occurred between days 1 and 2 and days 2 and 3, respectively. In square 8, one of the labelled progeny of division disappeared between days 4 and 5 and in square 3 the cell was lost by day 4. Note that cells in different squares migrated in different directions and that cells often changed direction.

Fernon, 1984) and flattening of the remaining cells; cell loss also tended to be greater in outer grid squares.

FGF induced marked changes in cell morphology (Fig. 6; Table 2). An increase in cell length occurred which was greatest between days 0 and 1, (possibly corresponding to the morphological changes associated with active migration described above) and between days 2 and 4 (Fig. 7). At this stage, the cells assumed a more regular elongated shape which was usually maintained throughout the culture period (Fig. 6; Table 2). This gradual elongation of cells into fibres was the major shape change in explants treated with FGF. Occasionally, cells began to elongate but then rounded up, for example, one of the progeny of the divided cell in Fig. 6. This also occurred in non-dividing cells and was usually a prelude to cellular disintegration.

There was an overall three-fold increase in cell length during the 5 day culture period in cells responding to FGF which did not divide (Fig. 7). The progeny of cells that underwent division during the culture period generally showed similar shape changes to non-dividing cells (Fig. 6). However, after day 1, cells that divided did not increase in length as much as cells that did not divide (P < 0.03; Fig. 7). This might be expected, as the progeny of a cell division are depleted in the process. Moreover, there was a significant difference in the degree of elongation of the two daughter cells arising from a single division (Table 2).

All cells in the lens epithelium, at least in the central region investigated, seemed to be capable of responding to FGF. Cell length and the degree of elongation, as well as cell migration (see above), were not dependent on the position of the injected cell within the explant. Moreover, at some stage during the culture period, every labelled cell in FGF-treated explants showed evidence of migration and elongation in excess of maximum values observed for cells in control explants.

Immunoneutralization studies
To confirm that bFGF is the molecule responsible for stimulating proliferation, migration and fibre differentiation, immunoneutralization studies were carried out. The bFGF-specific antibody neutralized the stimulatory influence of bFGF on cell proliferation and cell migration (Table 2). The 90–93% inhibition reported here compares well with the overall 93% inhibition of β-crystallin accumulation over a 5 day period reported...
Fig. 6. Diagram representing typical changes in the morphology of cells in explants cultured for 5 days in control medium or in medium supplemented with bFGF, 125 ng ml⁻¹.

Table 2. Cell elongation in control and bFGF-treated explants

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of cells</th>
<th>Elongation factor, day 5*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control:</td>
<td>29</td>
<td>1·4 (1·0–2·6)</td>
</tr>
<tr>
<td>FGF-treated:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No cell division</td>
<td>48</td>
<td>6·7 (1·8–17·5)</td>
</tr>
<tr>
<td>Cell division,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell A†</td>
<td>11</td>
<td>6·6 (1·7–15·0)</td>
</tr>
<tr>
<td>Cell B†</td>
<td>11</td>
<td>2·4† (1·2–4·3)</td>
</tr>
</tbody>
</table>

*Elongation factor = length of each cell divided by its width (for definitions, see Materials and methods). Each value represents the mean ± s.e.m. of the following numbers of individual cell measurements: controls, 29; FGF-treated, non-divided cells (■), 48; FGF-treated, progeny of cell divisions (○), 21–29. Only cells that survived the 5 day culture period were included. For definition of length, see Materials and methods.

Discussion

The initiation of synthesis of either β- or γ-crystallin can be used as a marker for lens fibre differentiation in mammals (see Introduction). Because β-crystallin is the first of these fibre-specific proteins to be synthesized during fibre differentiation (McAvoy, 1978; Campbell and McAvoy, 1984), it is the most convenient marker previously (Chamberlain and McAvoy, 1989). In the latter study, inhibition of [³H]thymidine incorporation measured at 40–48 h was only 68%, but a much higher concentration of bFGF was used.

Table 3. Inhibition of the action of bFGF by an antibody specific for bFGF

<table>
<thead>
<tr>
<th>Treatment</th>
<th>[³H]thymidine incorporation (disintegrations min⁻¹ explant):</th>
<th>Migration (µm/24 h):</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control†</td>
<td>0·45 ± 0·05</td>
<td>25·6 ± 4·4</td>
</tr>
<tr>
<td>FGF†</td>
<td>1·94 ± 0·24</td>
<td>69·5 ± 7·3</td>
</tr>
<tr>
<td>FGF+antibody¶</td>
<td>0·60 ± 0·18§</td>
<td>28·5 ± 7·2§</td>
</tr>
</tbody>
</table>

* Each value represents the mean ± s.e.m. of six determinations ([³H]thymidine incorporation) or 9–15 individual cell measurements (migration). The concentration of bFGF in the assay was 1 ng ml⁻¹ for thymidine incorporation, which was measured over a 21–24 h period, and 40 ng ml⁻¹ for migration, measured over a 0–24 h period.
† The medium contained non-immune IgG.
¶ The medium contained anti-FGF IgG.
§ This value is significantly lower than the corresponding value for FGF alone, P < 0·001.
for fibre differentiation in our experimental system. Separate studies have shown that FGF also induces y-crystallin accumulation in our epithelial explants (Chamberlain and McAvoy, 1987; Richardson and McAvoy, 1989). Under these conditions, cell elongation is also a marker for fibre differentiation; FGF-induced elongation has been studied by electron microscopy and the associated fine-structural changes have been shown to be analogous to changes characteristic of fibre differentiation in the intact lens (Lovicu and McAvoy, 1989). Thus, the morphological and molecular events induced by bFGF in our explants faithfully reproduce events in fibre differentiation in situ. All these events are also induced in lens epithelial explants by aFGF, but higher concentrations are required (Chamberlain and McAvoy, 1987, 1989; Lovicu and McAvoy, 1989). We used bFGF in this investigation because of its greater potency and its relative abundance in the neural retina, where it accounts for more than 99% of total FGF mitogenic activity and about 80% of FGF protein (Baird et al. 1985a). Moreover, it has recently been shown that the FGF-like activity of RCM described above (see Introduction) can be substantially blocked by an antibody to bFGF (Richardson and McAvoy, 1989).

This study provides information about the interrelation between the three major responses induced by bFGF in epithelial cells in lens explants: (a) proliferation, (b) migration and (c) fibre differentiation as shown by cell elongation and/or the accumulation of y-crystallin. We found that these three responses occurred sequentially as the concentration of FGF was increased, with half-maximal responses for proliferation, migration and fibre differentiation being achieved at 0.15, 3 and 40 ng ml

It was found that the anti-bFGF antibody previously shown to virtually completely inhibit the accumulation of y-crystallin and morphological changes characteristic of fibre differentiation had a comparable inhibitory effect on bFGF-induced cell proliferation and migration. These results are consistent with the suggestion that bFGF itself is the molecule responsible for inducing all three responses.

FGF has been shown to have multiple effects on other cell types. For example, bFGF is a potent stimulator of proliferation and chemotaxis in vascular endothelial cells and also induces plasminogen activator and collagenase production in these cells (Moscatelli et al. 1986; Presta et al. 1986; Saksela et al. 1988). These events are essential for neovascularization. FGF is also known to influence events in early development, notably the induction of mesodermal elements from ectoderm in amphibian embryos, and there is evidence that it has different inductive effects at different concentrations. Low levels induce ventral-type mesoderm, including mesenchyme, mesothelium and blood cells, whereas high levels of FGF induce greater muscle differentiation (Slack et al. 1987; Grunz et al. 1988). Kimelman and Kirschner (1987) recently provided evidence for the presence of mRNA for bFGF in Xenopus embryos, leading to suggestions that FGF acts as a morphogen responsible for inducing mesoderm during early amphibian development and together with other growth factors playing a critical role in determining the polarity of the amphibian embryo (Weeks and Melton, 1987; Slack et al. 1988).

The results of the present study may be relevant to our understanding of normal lens growth and development. The lens is surrounded by ocular media contained in three distinct compartments (Fig. 1). It is interesting to note that, in the lens epithelium, cell proliferation, fibre differentiation and possibly cell migration occur in distinct spatial patterns that appear to be related to the positions of these compartments, as follows: (a) The epithelial cells in the central region, which are bathed by the aqueous of the anterior chamber, do not undergo fibre differentiation and are non-proliferative or exhibit only very low levels of proliferation. (b) Epithelial cells at the lens equator and in the zone just anterior to it, the germinative zone, are exposed to the aqueous of the posterior chamber. In this region high levels of proliferation occur, but differentiation does not. (c) On the basis of autoradiographic studies of the lens in situ (Messier and Leblond, 1960; Hanna and O'Brien, 1961; Brolin et al. 1961), Hanna and Keatts (1966) have suggested that movement of epithelial cells from the anterior to the posterior of the lens during normal growth involves active migration of cells at the equator, that is, near the junction between the aqueous of the posterior chamber and the vitreous. (d) Cells below the equator, which are exposed to the vitreous, undergo elongation and differentiate to become fibre cells.

Given the concentration-dependent three-fold response of lens epithelial cells to bFGF reported in the present study, it can be seen that the spatial patterns of cellular behaviour described above are consistent with the existence of a gradient of concentrations of FGF in the ocular media, ranging from negligible amounts in the aqueous of the anterior chamber to concentrations in the vitreous sufficient to promote lens fibre differentiation. The literature contains other data consistent with this suggestion. For example, it seems likely that the retina, which is in close proximity to the vitreous, is a major source of FGF in the eye (Barrault et al. 1981; Baird et al. 1985a; Courty et al. 1985). Moreover, FGF has been isolated from chick embryo vitreous (Mascarello et al. 1987) and concentrations of 10-20 ng ml

Whilst acknowledging that other interpretations of the data are not precluded at this stage, the results of the present study do raise the interesting possibility that variations in the FGF concentration in the different...
ocular media that bathe the lens may provide positional information and determine spatial patterns of proliferation, migration and fibre differentiation in the lens in situ.

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References


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