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Factors modulating mouse lens epithelial cell morphology with differentiation and development of a lentoid structure *in vitro*

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Summary

The morphological and cellular changes that occur with differentiation and development of a lentoid structure from cultured mouse lens epithelial cells have been found to be dependent on the presence of lens capsule in association with the cells. The development of the 'lentoid body' is a multiphase process involving cell replication, synthesis of mucosubstances and a basement collagen membrane, cell aggregation and differentiation. Stage-specific synthesis of lens proteins confirms that the genes regulating normal differentiation *in vivo* are operating in the *in vitro* system.

The hydrated collagen gel studies described in this report demonstrate that the cuboidal morphology and apical–basal polarity of the lens epithelial cells are dependent on their relationship with the lens capsule. Following a replicative phase the cells assume a mesenchyme-like morphology and migrate into the gel. Trypsinized cells freed from the lens capsule replicate but form colonies on the surface of the gel. The implications of these results are discussed with respect to previous observations made on normal lens development and the abnormalities associated with the congenital cataractous embryonic lens.

Key words: mouse, lens, epithelial cell, lentoid-body, proteins.

Introduction

The normal lens is a transparent, nonvascularized tissue encapsulated by a basement membrane composed of collagen, glycoproteins and proteoglycans (Kefalides, Alper & Clark, 1979). It has been proposed that changes in the glycoprotein concentration of the extracellular matrix influence early lens differentiation *in vivo* (Hendrix & Zwaan, 1974). During development of the embryonic lens the cells undergo dramatic changes in growth, morphology, cell interactions and gene expression. The central lens epithelial cells subjacent to the collagen lens capsule synthesize specific lens proteins, the α- and β-crystallins. The elongated fully differentiated fibre cells synthesize an additional class of proteins, the γ-crystallins (Papaconstantinou, 1967; Harding & Dilley, 1976; McAvoy, 1980; Piatigorsky, 1981). The capsule proteins and sulphated mucopolysaccharides of the collagen lens capsule are synthesized by both epithelial and fibre cells (Rafferty & Goosens, 1978), and are deposited in layers originating from the cell surface (Heathcote, Bailey & Grant, 1980).

The monolayer of cuboidal lens epithelial cells that overlies the fibres and is subjacent to the lens capsule, has a dorsal–apical polarity, and becomes inhibited in the G_1 phase of the cell cycle *in vivo* after birth. However, the cells maintain the ability to replicate throughout life if stimulated by wounding or cell culture (Muggleton-Harris, 1971*a*,*b*, 1972; Nelson & Rafferty, 1976; Muggleton-Harris, Lipman & Kearns, 1981). Cultured mouse lens epithelial cells have a finite and well-characterized population doubling level (PDL), maintain a diploid set of chromosomes, are contact inhibited and grow as a monolayer (Muggleton-Harris *et al.* 1981; Lipman & Muggleton-Harris, 1982; Muggleton-Harris & Higbee, 1986).

In vitro studies have demonstrated that 'lentoid bodies', which have similar characteristics to the embryonic lens *in vivo*, can be induced to differentiate from presumptive lens ectoderm by a variety of 'unspecific stimuli' (Karkinen-Jääskeläien, 1978). We have previously reported that fragments of lens capsule can promote the differentiation of a 'lentoid body' in cultured cloned mouse lens epithelial cells (Muggleton-Harris *et al.* 1981). The morphogenesis of

the lens-like structure *in vitro* provides a system that can readily be analysed with regard to factors influencing or regulating cell interactions and behaviour during development and differentiation. Specifically, cultured mouse lens epithelial cells can be monitored during the multiphase process of 'lentoid body' development for the synthesis of capsular proteins and lens crystallins.

The maintenance of an apical–basal polarity of the epithelial cell monolayer in relationship to the collagen capsule is an important aspect of cell regulation. Disruption of this normal arrangement can result in cell replication, disturbance of the fibres and gross abnormalities of the lens structure. Such disturbances are found in the developing lens of congenital cataractous mutants (Muggleton-Harris, 1986; Muggleton-Harris & Higbee, 1986). In this paper we also describe the behaviour of mouse lens epithelial cells when grown on hydrated collagen gels with or without the collagen lens capsule present.

Materials and methods

Mouse lens epithelial cultures

Complete details for the culture of mouse lens epithelial cells (MLE) have been fully described (Muggleton-Harris *et al.* 1981; Muggleton-Harris & Higbee, 1986). In brief, adult mice were killed by cervical dislocation, the eyes removed and washed through alternate rinses of 70% EtOH and modified Earle's medium with Hanks' salts (HMEM), plus antibiotics. The lens was dissected free of the eye and the lens capsule with its adhering monolayer of epithelial cells was removed from the lens fibres. The cells are then trypsinized free from the capsule and used to establish the cell cultures.

Cultured MLE replicate and form a monolayer, they have a well-defined and limited life span. Clonal studies have shown a heterogeneity between individual clones but normal MLE have an average population doubling level (PDL) of 8. The cell size, nucleocytoplasmic ratio and nuclear diameter alter with successive PDL. The cells are stellate and have active ruffling of membranes and cytoplasmic processes during the growth phase. When cell replication ceases, a quiescent period follows during which intracellular changes are seen e.g. increase in lysosomal vacuoles and granulation of the cytoplasm. Despite these intercellular changes normal MLE can remain in this phase for periods in excess of four months before phasing out by cytolysis, 'lentoid bodies' do not form in these cultures. However, if a piece of cell-free collagen capsule is placed on top of a monolayer of cultured MLE, a series of changes in morphology and behaviour of the cells is noted which results in the development of a 'lentoid body' (Muggleton-Harris *et al.* 1981).

Preparation of antisera to the lens proteins

Rabbit antisera against the α, β and γ fractions of the mouse lens proteins were prepared using peak fractions of these crystallins as separated by gel filtration on a LKB ALA22 column. The buffers and methods for separation, fractionation and characterization of lens crystallins have been described in full (de Pomerai, Pritchard & Clayton, 1977; Clayton, Campbell & Truman, 1968; Pal & Modak, 1984; McAvoy, 1978). Antisera against total lens proteins, and the α, β and γ fractions were prepared by injecting $10\,mg\,ml^{-1}$ total soluble lens protein emulsified with an equal volume of Freunds complete adjuvant subcutaneously into separate rabbits at weekly intervals for six weeks. The rabbits were bled prior to the injections to obtain preimmune sera. Subsequently the rabbits received three booster shots at monthly intervals and bled 10 days after the booster shots for the sera.

SDS–polyacrylamide gel electrophoresis (SDS–PAGE) was carried out in a slab gel apparatus using 15% running gel and 4–5% stacking gel as described by Pal & Modak (1984). A set of standard relative molecular mass (M_r) marker proteins was used to calibrate the gel and M_r of the protein bands.

Immunoelectrophoresis was carried out on glass slides coated with agarose (Pharmacia Agarose A) in Tris–glycine buffer, for 2 h at 250 v at 6°C. Antiserum to the crystallins was loaded in a trough cut parallel to the electrophoretic axis and the glass slides were kept in a moist chamber for diffusion, washed exhaustively in 1% saline, dried and stained with 0·5% Coomassie brilliant blue in acetic acid:ethanol:water (10:35:55), (Pal & Modak, 1984). Ouchterlony gels were also run to verify the specificity of the sera to the crystallin fractions against each other and the preimmune sera (Ouchterlony, 1949, 1953; Ringens, Liem-The, Hoenders & Wollensak, 1978).

Protein synthesis of lens cells

Fluorescent isothyanate-conjugated goat anti-rabbit IgG serum was used to visualize the localization of rabbit sera against the specific crystallins in prepared sections of Carnoy's fixed lens material (McAvoy, 1978; Zwaan, 1968; Muggleton-Harris & Higbee, 1986), and in ethanol-fixed cultured MLE cells (Creighton, Mousa, Miller, Blair & Trevithick, 1981). An epifluorescent attachment on a Leitz Ortholux inverted microscope was used to study cells at various stages of proliferation and differentiation for changes in the morphology and cellular behaviour with specific lens crystallin synthesis.

Presence of mucosubstances and collagen components in vitro

Cultured MLE were examined at the various phases of their growth and differentiation for the presence of those components that are synthesized to form the collagen lens capsule *in vivo*. Weakly acidic sulphated mucosubstances hyaluronic and siolomucins were identified by staining with Alcian blue (pH2·5–1·9). Massons trichrome stain detected the presence of collagen and intercellular fibres in the cell cultures (Luna, 1960).

Collagen gels

Collagen gel membranes were made by modifying the methods of Michalopoulos & Pitot (1975). 0·6 ml of collagen was poured into a multiwell plastic plate, the

diameter of the wells was 23·5 mm. 70 μl of FBS was added to each well plus 134 μl of a 2:1 mixture of 10 × HMEM and 0·34 M-NaOH. The collagen plus additives are well mixed then allowed to set. The gels were washed with two changes of HMEM plus FBS to allow them to equilibrate to the correct pH of 7·2. The equilibration takes place in a 37°C, 5% CO_2 humidified incubator for 1½ h.

By modifying a procedure used for chick lens material (Greenberg & Hay, 1982), small segments (0·1–0·3 mm) of lens capsule with its adhering lens epithelial cells were excised from the central anterior area of a freshly isolated lens capsule. The segments were placed cell side down on to the gel membrane and 1 ml of HMEM plus FBS and antibiotics added gently to the well. Other gels receive a single cell suspension of freshly trypsinized lens epithelial cells. The gels plus cells and/or capsule were placed in the incubator for 24 h. The gels are then carefully detached from the well surface and allowed to float in fresh medium for a period of 4 days. The amount of medium was reduced and the gels plus cells were then fixed with 70% EtOH and stained with Giemsa solution overnight. The gels were mounted on a microscope slide with a coverslip sitting on small ridges of wax to prevent compaction or distortion of the gel membrane.

Photomicroscopy

Leitz Diavert inverted microscope with an epifluorescent attachment was used for the photographs taken of the FITC crystallin cell studies. Phase optics provided the pictures of the cell membrane and lentoid body development.

Results

The synthesis of the specific proteins associated with the lens epithelial cells *in vivo* has been confirmed for cloned MLE *in vitro*. Fig. 1A shows a clone of MLE 8 days after the initial cell was isolated, the cytoplasm is positive for the presence of the lens proteins, the nucleus negative. Fig. 1B shows a similar clone of cells with antisera to the γ-crystallins used on them, they are negative for this class of proteins which is associated with the differentiated fibre cells *in vivo*. A monolayer of MLE cells is formed from cloned cells, Fig. 2A shows a culture 15–20 days after the cells' initial isolation, the cells have replicated approximately 8 population doubling levels (PDLs). They have formed a typical epithelial cell sheet with close cell contacts, an accumulation of lysosomal vacuoles in the perinuclear area can be seen in some cells, these increase towards the end of a cell's finite lifespan that occurs at 8–12 PDLs. The cells at the perimeter of the monolayer have ruffling membranes and active filopodia. A piece of collagen lens capsule free of cells has been placed on top of the monolayer and can be seen crossing the centre of the photograph.

Within 14–25 days following the addition of the piece of collagen, subpopulations of the cells are shown to synthesize a layer of mucosubstances, hyaluronic acids and siolomucins. Fig. 2B is a transmitted light photograph of the culture previously shown in Fig. 2A. Fig. 3A shows a similar culture stained with Alcian blue to identify the areas where the mucin layer is formed by the cells. The stained nuclei of the cells comprising the cell sheet show that in certain places the cells are forming foci and these are associated with those areas where mucin is found. Fig. 3B shows a cross section through the monolayer of cells and Fig. 3C an area where the cells have formed a focus. Cells that do not form the mucin–cell complex continue to follow the normal *in vitro* pattern of replication then phasing out and dying by cytolysis. Within 6–10 days of the mucin-layer phase the presence of collagen can be detected with Massons trichrome stain in the areas where the cells are forming foci, Fig. 4. If the culture is left undisturbed for a further 20–30 days the collagenous mucin membrane forms a network within which the cells become heavily concentrated. Fig. 5A shows an enlargement of such an area where the collagen cell areas form large attenuated processes and the aggregates of cells within the processes when stained with Massons trichrome. The area where the 'lentoid body' is forming is stained very heavily and appears quite black. Fig. 5B shows a cell–collagen sheet of a culture 65 days after initiation from the clone of cells. The edge of the cell–collagen layer has been brought into focus, also a well-formed 'lentoid body' can be clearly seen. The cells are aligned along the clearly delineated edge of the collagen membrane and a focus of cells leads away from the edge. When stained with Massons trichrome this area stains positively in a similar manner to that already shown in Fig. 4. If antiserum to the γ-crystallins is used at this stage, the cells positive for these proteins are clearly seen along the membrane perimeter, in the foci of cells leading away from the membrane, and the fully formed 'lentoid body' is positive, Fig. 5C. The results from these studies describe the multiphase process of induction of a 'lentoid body' from cultured cloned mouse lens epithelial cells. The process takes approximately 12 weeks and is induced by placing a piece of cell-free collagen lens capsule on top of a monolayer of cells. If the collagen is not present the cells will follow their normal well-characterized *in vitro* lifespan and phase out by cytolysis without further differentiation. The experiments with the hydrated gels have shown that the cell–basement membrane relationship is important for regulating the behaviour and morphology of cultured MLE. Fig. 6 describes the changes observed in numerous collagen gels, because of the three-dimensional aspects of those studies it was difficult to take clear

photographs of the cells themselves. The MLE attached to the lens capsule replicate and then very thin long mesenchyme-like cells are seen to penetrate deep into the hydrated collagen gel. The cells exhibit a leading pseudopodium with filopodial extensions and a trailing process. The trypsinized MLE replicate and form tightly regulated colonies on the surface of the gels, there are no mesenchyme-like cells seen in these preparations. When the cells comprising these colonies are counted they have a range from 2–38 cells in each colony. This is similar to the clonal distribution pattern we previously reported for cultured clones of MLE.

Discussion

In this report we describe the specific stimulus of a differentiated lentoid structure formed from cultured mouse lens epithelial cells. The multiphase process of response by subpopulations of the cell monolayer involves the synthesis of components of the lens capsule. First, the lens epithelial cells produce mucosubstances and then a collagen basement membrane. Although 'lentoid bodies' have been described in cultured lens epithelial cells previously, the specific stimulus and cellular changes in the development of these structures were not identified. The anterior lens

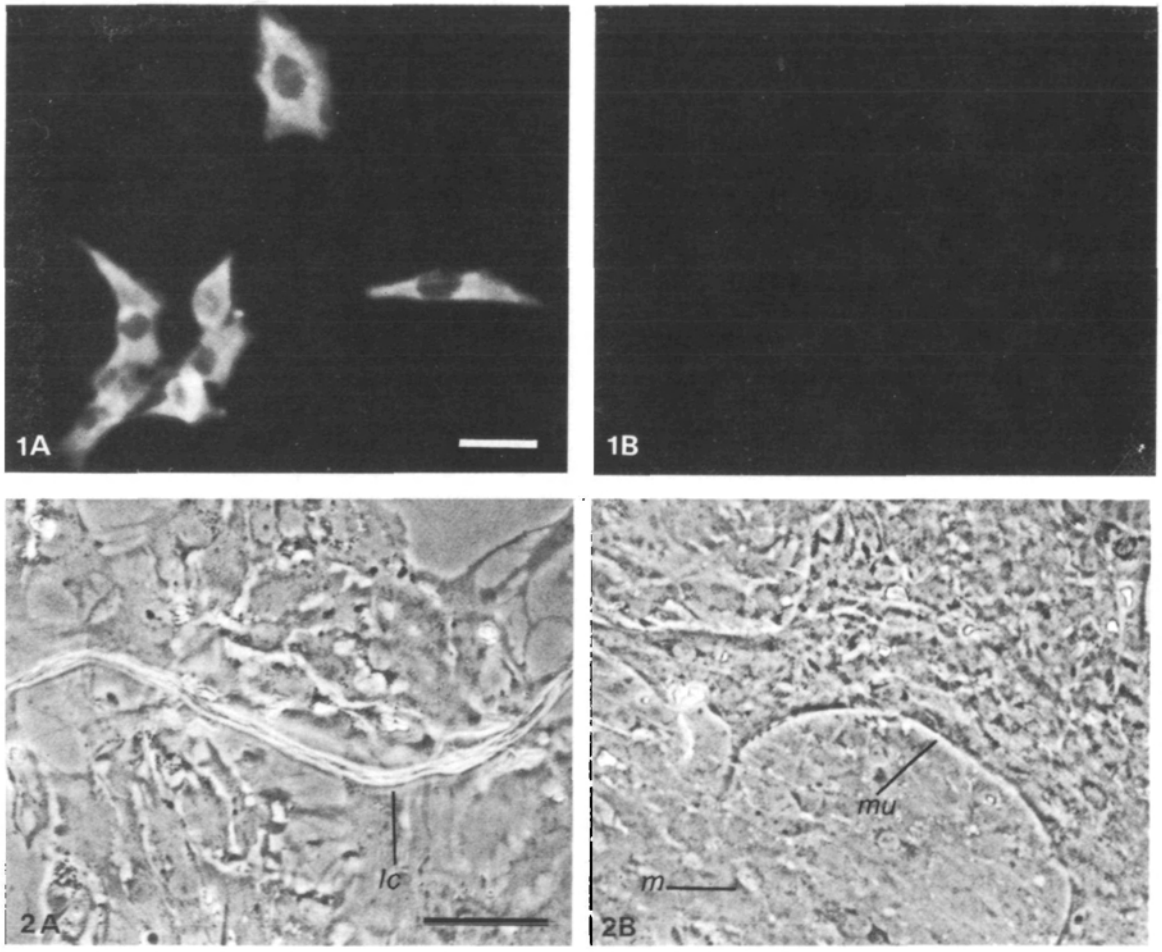

Fig. 1. (A) A clone of cultured mouse lens epithelial cells (MLE) is shown to be synthesizing the α- and β-crystallins, the initial cell was identified 8 days previously. The cells have the typical morphology for cloned MLE and demonstrate a positive fluorescence in the cytoplasm. (B) A similar-sized clone of MLE exposed to antisera specific for γ-crystallins. The cells show no positive fluorescence for this class of lens protein which is normally associated with the fully differentiated fibre cell population of the lens *in vitro*. Bar, 20 μm.

Fig. 2. (A) A monolayer of MLE grown from cloned cells, the culture is approximately 15–20 days from initiation, the cells have replicated 8 population doubling levels. Ruffling membranes and active filopodia can be seen in the cells at the edge of the culture. A piece of collagen lens capsule free of cells has been placed in contact with the cell sheet, the refractive thread of lens capsule material can be seen running across the middle of this photograph (*a*).
(B) A monolayer of MLE 22 days following the addition of the collagen material to the culture. A layer of mucosubstances, hyaluronic acids and siolomucins has been synthesized by a subpopulation of the cultured cells. The photograph has been chosen to demonstrate the monolayer of cells (*m*) and an area of the outline of the mucin layer (*mu*). Bar, 50 μm.

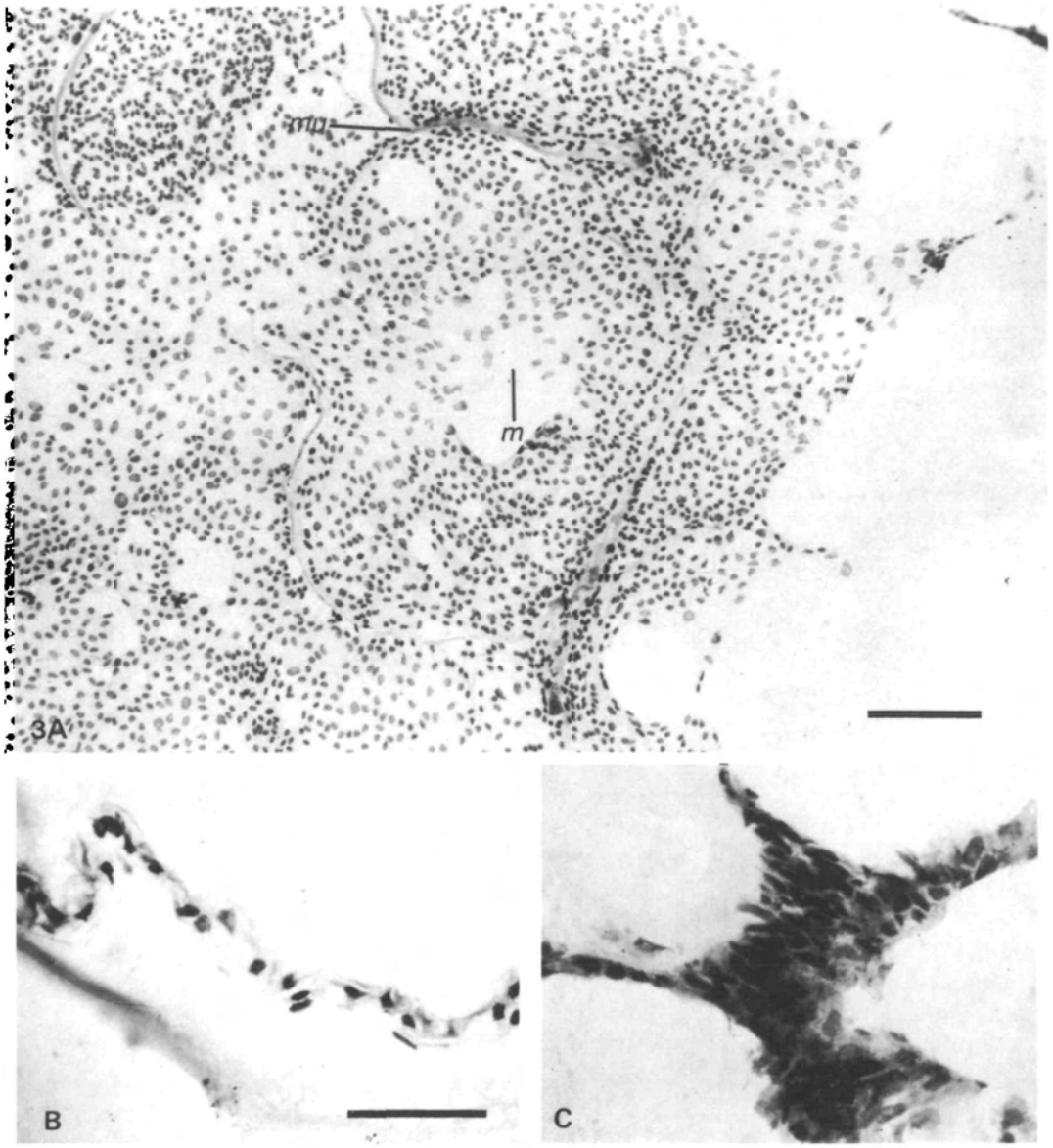

Fig. 3. (A) A culture of the same age and phase of proliferation as that shown in Fig. 2B. The preparation has been fixed and stained to show the nuclei of each cell. Alcian blue stain demonstrates the presence of the mucosubstances in this culture and the outline of the layer can be detected in an area free of cells within the monolayer cell sheet (*m*). The stained nuclei also demonstrate the patterns or foci of cells that form along the edges of the mucin layer (*mu*). (B) A cross section through a cell monolayer at the same stage as that shown in Fig. 3A. The monolayer was detached from the dish and embedded, sectioned and stained. The nuclei of the cells and the associated mucin layer can be seen. (C) An area of the same preparation as in Fig. 3A where the cells are concentrating along the edge of the mucin layer. Bars, (A) 200 μm; (B,C) 50 μm.

epithelial cells *in vivo* behave very similarly to the MLE *in vitro* in that they replicate to form a contact-inhibited monolayer. If stimulated they synthesize components of the collagen lens capsule. The process of differentiation into fibres and formation of the 'lentoid body' occurs when the cells and the mucin–collagen basement membrane form specific foci. *In vivo* a small population of the epithelial cells located at the germinative zone elongates and forms the fibres that constitute the lens. The synthesis of the final class of crystallins has been shown to be turned on cell by cell as the cells begin their process of differentiation and elongation (Zwaan, 1968; Zwaan & Hendrix, 1973). The synthesis of γ-crystallins in our cultures first occurs in specific areas along the cell–basement membrane complex and then in the cells forming the lentoid structures. The concentration of the crystallins was not determined during

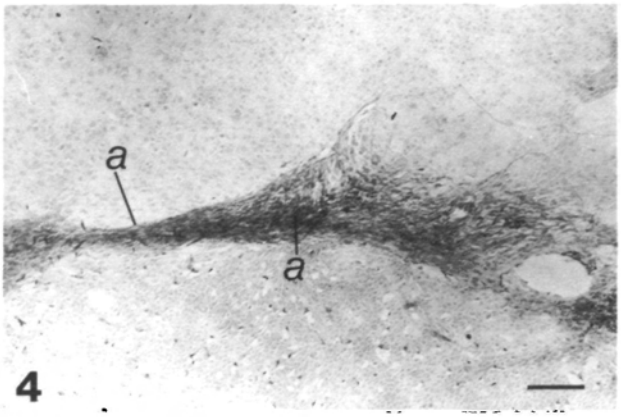

Fig. 4. A culture of MLE 10 days later than the phase shown in Fig. 3A. This preparation has been fixed and stained with Massons trichrome stain which demonstrates the presence of collagen in the cultures. The cell foci or aggregation patterns are very distinctive at this stage (*a*). Bar, 200 μm.

these experiments, however, it will form part of the future studies on the morphology and protein synthesis of the lentoid bodies themselves. It has been shown that the proportion of crystallins in lentoid-containing cultures does not resemble those of embryonic lens fibres (de Pomerai *et al.* 1977). The *in vitro* system described here will permit a more detailed analysis of the cellular and biochemical changes at the cellular level with differentiation and development of lentoid structures.

The hydrated collagen gel studies have demonstrated the importance of the cells relationship to the basement membrane in regulating replication and morphology. The behaviour of the mesenchyme-like cells in this system supports the ideas presented by investigators using bovine lens cells. The role of the substratum in cell shape variations has been demonstrated as important for cell differentiation and mitosis (Iwig & Glaessar, 1979), and cell behaviour (Elsdale & Barch, 1972). Although observations on the migration of mesenchyme-like cells using avian lens material have been described previously (Greenburg & Hay, 1982). The behaviour of mouse lens epithelial cells is of specific interest to us because some of the characteristics noted in this work could be important for our studies on the pathogenic process resulting in a congenital cataractous lens (Muggleton-Harris & Higbee, 1986). Factors that affect loss of cell regulation resulting in replication and abnormalities in the lens structure during embryogenesis *in vivo*, may now be studied *in vitro* using lens epithelial cells from congenital cataractous mutants (Muggleton-Harris, 1986).

It is interesting to note that in a recent report by Greenburg & Hay (1986) the morphology of avian

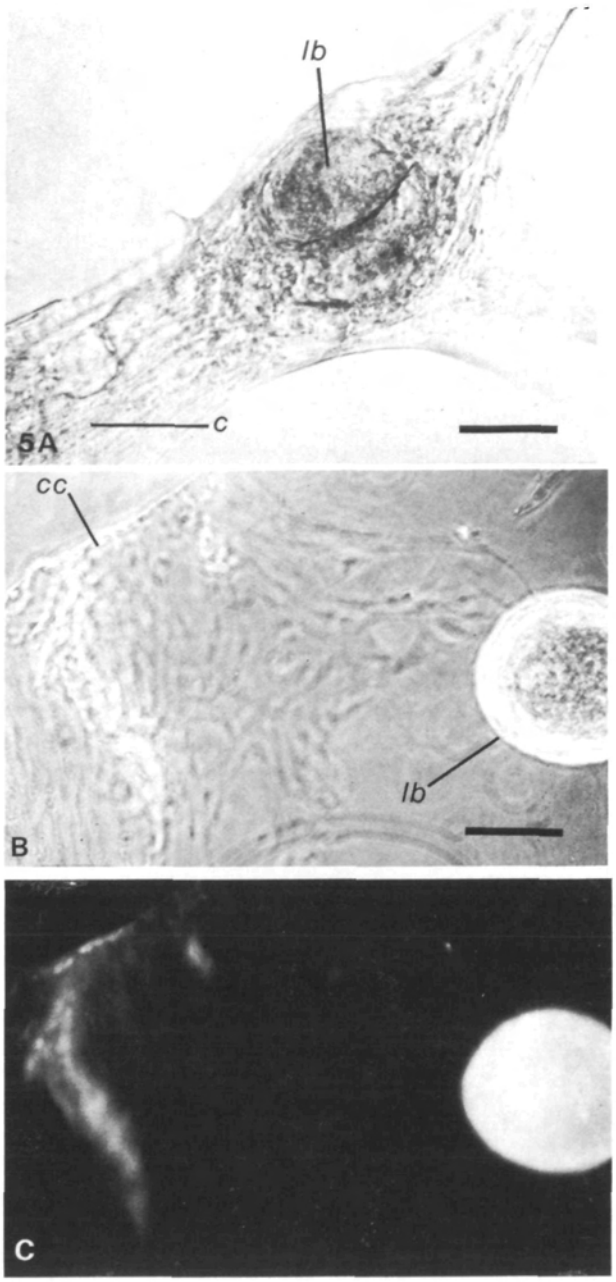

Fig. 5. (A) A culture of MLE 25 days following the detection of the mucin–collagen components. The culture is now composed of a network of long attenuated collagen processes (*c*), the cells that lie within these processes are more heavily concentrated in specific areas. The cells that are forming the early 'lentoid body' can be seen quite clearly (*lb*). (B) A culture of cells 65 days after its initiation from a clone of cells. The edge of the cell–collagen sheet has been brought into focus (*cc*), as well as the fully formed 'lentoid body' (*lb*). (C) Antiserum specific to the γ-crystallins has been used on the preparation shown in Fig. 5B. The cells aligning the edge of the collagen–cell layer and the spur of cells are positive for this specific differentiated lens protein associated with the lens fibres, individual cells can be detected along the membrane edge. The fully formed 'lentoid body' is also positive for the γ-crystallins. Bars, 125 μm.

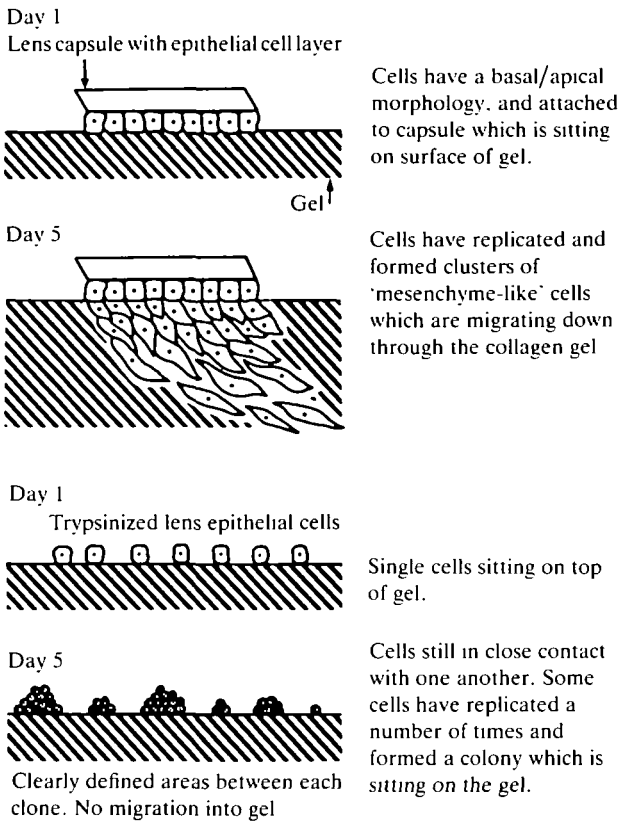

Fig. 6. A diagrammatic representation of the behaviour of cultured mouse lens epithelial cells on a hydrated collagen gel. One method allows the cells to remain attached to the lens capsule, the other uses a single cell suspension. The cells were cultured for 5 days prior to fixation and staining.

mesenchyme-like cells in collagen gels is indistinguishable from 'true' mesenchyme cells at the light and ultrastructural level. The newly formed mesenchyme-like cells lack the ability to synthesize lens-specific δ-crystallins, type IV collagen and laminin. When these cells are removed from the gel and replated on two-dimensional surfaces, they remain bipolar and will invade collagen matrices. We have observed (Muggleton-Harris & Higbee, 1986) that the cells that penetrate the collagen capsule in the posterior area of the congenital cataractous lens are similar in morphology to the migrating MLE within hydrated collagen gels. Detailed SEM and ultrastructural studies are presently underway to study the mouse lens epithelial cells during the phases of replication, migration and differentiation described in this paper.

References

Clayton, R. M., Campbell, J. C. & Truman, D. (1968). A re-examination of the organ specificity of lens antigens. *Expl Eye Res.* **7**, 11–29.

Creighton, M. O., Mousa, G. Y., Miller, G. G., Blair, D. G. & Trevithick, J. R. (1981). Differentiation of rat lens epithelial cells in tissue culture. IV. Some characteristics of the process including possible 'in vitro' models for pathogenic process in cataractogenesis. *Vision Res.* **21**, 25–35.

de Pomerai, D. I., Pritchard, D. J. & Clayton, R. M. (1977). Biochemical and immunological studies of lentoid formation in cultures of embryonic chick neural retina and day old chick lens epithelium. *Devl Biol.* **60**, 416–427.

Elsdale, T. & Bard, J. (1972). Collagen substrata for studies on cell behaviour. *J. Cell Biol.* **54**, 626–637.

Greenburg, G. & Hay, E. D. (1982). The transformation of epithelium to mesenchyme-like cells with a hydrated collagen lattice. *Anat. Rec.* **202**, 69A.

Greenburg, G. & Hay, E. D. (1986). Cytodifferentiation and tissue phenotype change during transformation of embryonic lens epithelium to mesenchyme-like cells *in vitro*. *Devl Biol.* **115**, 363–380.

Harding, J. J. & Dilley, K. H. (1976). Structural proteins of the mammalian lens: A review with emphasis on changes in development, aging and cataract. *Expl Eye Res.* **22**, 581–595.

Heathcote, J. G., Bailey, A. J. & Grant, M. E. (1980). Studies on the assembly of the rat lens capsule: Biosynthesis and partial characterization of the collagenous components. *Biochem. J.* **176**, 283–294.

Hendrix, R. W. & Zwaan, J. (1974). Changes in the glycoprotein concentration of extracellular matrix between lens and optic vesicle associated with early lens differentiation. *Differentiation* **2**, 337–362.

Iwig, M. & Glaessar, D. (1979). On the role of the substratum in cell shape variations of Bovine lens cells. *Ophthalmic Res.* **11**, 298–301.

Karkinen-Jääskeläinen, M. (1978). Permissive and directive interactions in lens induction. *J. Embryol. exp. Morph.* **44**, 167–179.

Kefalides, N. A., Alper, R. & Clark, C. C. (1979). Biochemistry of basement membranes. *Int. Rev. Cytol.* **61**, 167–228.

Lipman, R. D. & Muggleton-Harris, A. L. (1982). Modification of the cataractous phenotype by somatic cell hybridization. *Som. Cell Genet.* **8**, 791–800.

Luna, L. G. (1960). Methods for connective tissue. In *Manual of the Histological Training Methods for the Armed Forces Inst. of Pathology*, pp. 72–100. New York: Blakiston Div., Mc-Graw-Hill Book Company.

McAvoy, J. W. (1978). Cell division, cell elongation and distribution of α, β and γ crystallins in rat lens. *J. Embryol. exp. Morph.* **44**, 149–165.

McAvoy, J. W. (1980). Induction of the eye lens. *Differentiation* **17**, 137–149.

Michalopoulos, G. & Pitot, H. C. (1975). Primary culture of parenchymal liver cells on collagen membranes, morphological and biochemical observations. *Expl Cell Res.* **96**, 70–78.

Muggleton-Harris, A. L. (1971*a*). Cellular events concerning the developmental potentiality of the transplanted nucleus with reference to the aging lens cells. *Expl Gerontol.* **6**, 279–285.

MUGGLETON-HARRIS, A. L. (1971*b*). Aging factors affecting the ability of adult lens cell nuclei for cleavage and development. *Expl Gerontol.* **6**, 461–467.

MUGGLETON-HARRIS, A. L. (1972). Aging effects at the cellular level, studied by transferring nuclei from organ cultured lens cells. *Expl Gerontol.* **7**, 219–225.

MUGGLETON-HARRIS, A. L. (1986). Mouse mutants, model systems to study congenital cataract. In *Int. Rev. Cytol.* **104**, 25–36.

MUGGLETON-HARRIS, A. L., LIPMAN, R. D. & KEARNS, J. (1981). *In vitro* characteristics of normal and cataractous lens epithelial cells. *Expl Eye Res.* **32**, 563–573.

MUGGLETON-HARRIS, A. L. & HIGBEE, N. (1986). *In-vitro* and *in-vivo* characterization of the Lop mutant lens. *Expl Eye Res.* (submitted).

NELSON, K. J. & RAFFERTY, N. S. (1976). A scanning electron microscope study of the lens fibres in healing mouse lens. *Expl Eye Res.* **22**, 335–346.

OUCHTERLONY, O. (1949). Antigen-antibody reactions in gels. *Acta pathol. Microbiol. Scand.* **26**, 507–519.

OUCHTERLONY, O. (1953). Antigen-antibody reaction in gels. IV. Types of reactions in co-ordinated systems of diffusions. *Acta pathol. Microbiol. Scand.* **32**, 231–240.

PAL, J. K. & MODAK, S. P. (1984). Immunochemical characterization and quantitative distribution of crystallins in the epithelium and differentiating fibre cell population of chick embryonic lens. *Expl Eye Res.* **39**, 415–434.

PAPACONSTANTINOU, J. (1967). Molecular aspects of lens cell differentiation. *Science* **156**, 338–346.

PIATIGORSKY, J. (1981). Lens differentiation in vertebrates: A review of cellular and molecular features. *Differentiation* **19**, 134–153.

RAFFERTY, N. S. (1973). Experimental cataract and wound healing in mouse lens. *Invest. Ophthamol.* **12**, 156–160.

RAFFERTY, N. S. & GOOSENS, W. (1978). Growth and aging of lens capsule. *Growth* **42**, 375–389.

RINGENS, P. J., LIEM-THE, K. W., HOENDERS, H. J. & WOLLENSAK (1978). Normal and cataractous human eye lens crystallins. In *Lens Aging and Development of Senile Cataracts. Interdis. Topics in Gerontol.* vol. 122 (ed. O. Hockwin & S. Karger), pp. 193–211. Basel, Switz.

ZWAAN, J. (1968). Lens specific antigens and cytodifferentiation in the developing lens. *J. Cell Physiol.* **72**, 47–72.

ZWAAN, J. & HENDRIX, R. W. (1973). Changes in cell and organ shape during early development of the ocular lens. *Am. Zool.* **13**, 1039–1049.

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