Electron microscopy of cultured mammalian lenses

I. Initial changes which precede and accompany the stimulation of DNA synthesis and mitosis

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The changes in tissue organization which precede and accompany DNA synthesis and mitosis in rabbit lenses maintained in organ culture were characterized at the ultrastructural level. Lenses were cultured in media which are known to trigger cell division and compared with lenses cultured in media which retain the central epithelium in a state of nonproliferation. Since DNA synthesis and mitosis follow a specific spatiotemporal pattern, specific regions of the epithelium were evaluated at various times of culture. At 1 hour the basal region of the epithelial cells was devoid of the intercellular spaces which typified the 0 hour preparations. Sections from the peripheral region at 3 hours showed extensive and pronounced infoldings of the cell membrane. At 7 hours there was an increase in the number of free ribosomes. After 15 hours intercellular spaces similar to those at 0 hours were observed, the peripheral epithelium was multilayered, and the cells were noticeably elongated. These changes precede DNA synthesis and mitosis which commence at 22 and 35 hours, respectively. Several of the changes in tissue organization were first noted in the periphery of the lens and subsequently in the central region, and they suggest a relationship between the spatiotemporal pattern of DNA synthesis and mitosis and prior ultrastructural modifications. These changes and additional findings at 22, 31, and 48 hours are correlated with recent observations on the pattern of macromolecular synthesis in the cultured lens. The role of tissue organization and modifications in cell-to-cell relationships in the control of mitosis is discussed.

Key words: crystalline lens, tissue culture, DNA, biosynthesis, mitosis, electron microscopy, ultrastructure, time factors, cell division, tissue organization, cell-to-cell relationships, organ culture.

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Cell division in the adult lens is primarily restricted to a peripheral region of the epithelium referred to as the germinative zone. The cells anterior to the germinative zone are normally devoid of proliferative activity and are blocked in the G-1 phase of the cell cycle (G-0 state). However, upon isolation and organ culture...
in certain media or following a mechanical injury to the lens in vivo, the central epithelial cells can be induced to enter DNA synthesis and mitosis. It is also possible to culture the lens under conditions in which the central epithelial cells retain the block-to-cell division. The epithelium of rabbit lenses maintained in such media can be triggered to enter the cell cycle by the addition of rabbit serum to the medium. Lenses cultured in media containing rabbit serum commence DNA synthesis at 22 to 24 hours and mitosis at about 33 to 35 hours. It has also been shown that the initiation of mitosis in the cultured lens is preceded by and dependent upon characteristic and well-defined periods of intensive RNA and protein synthesis. The entire proliferative response follows a specific spatiotemporal pattern. For example, cells engaged in DNA synthesis at 22 to 24 hours are located immediately anterior to the germinative zone. Subsequently, cells in DNA synthesis are found throughout the central epithelium. Mitosis follows a similar spatiotemporal pattern.

It would seem that the mechanism(s) which maintains the central epithelial cells in "G-0" or which accompanies the release of the cells from this state must have a basis in the over-all organization of the tissue. Changes in tissue organization have been suggested to play an important role in the control of cellular proliferation and may be a reflection of the trigger mechanism of cell division itself. It would certainly seem that if the phenomena of contact inhibition or cellular communication play a role in the control of cell division, the triggering of the cell cycle should be accompanied by changes in cell-to-cell relationships which would perhaps best be observed under the electron microscope.

Several investigators have characterized the fine structure of the lens under normal and pathologic conditions. However, little information is available concerning changes in tissue organization and in the fine structure of the epithelial cells which foreshadow the onset of the cell proliferation under in vivo or in vitro conditions. The lens is amenable to such studies since it is a highly organized tissue and, because of several unique properties, i.e., avascularity, lack of innervation, and enclosure within the lens capsule, it can be isolated and maintained in organ culture with a minimal change in tissue organization. Moreover, studies concerned with the role of tissue organization in the control of cell division are not possible when the usual techniques of cell culture are used, since the method itself destroys the tissue organization which we are attempting to study.

This paper is intended to characterize the changes in tissue organization which precede the initiation of mitosis in the cultured rabbit lens. The fine structure of these lenses is compared with that of lenses which have been cultured in a medium which is known to maintain the central epithelium in the G-0 state. Since mitosis follows a specific spatiotemporal pattern, emphasis has also been placed on an analysis of specific regions of the tissue at various times of culture.

Materials and methods

New Zealand white rabbits 7 to 9 weeks of age were used throughout this study. The animals were killed by an air embolism. The eyes were enucleated, and the lenses were isolated and cultured as previously described. In the first group of experiments, one lens from each animal was fixed immediately after isolation in a 1 per cent OsO₄ solution prepared in Millonig phosphate buffer, pH 7.3. These lenses were designated 0 hour lenses. The contralateral lenses were cultured for various times in medium 199 (Microbiological Associates, Bethesda, Md.) containing 23 per cent (v/v) rabbit serum. This medium is known to induce a marked mitotic response. In additional studies one lens from each animal was cultured in medium 199 containing 23 per cent (v/v) rabbit serum dialysate (Colorado Serum Co., Denver, Colo.) or solely in medium 199. The contralateral lenses were cultured for various times in medium 199 (Microbiological Associates, Bethesda, Md.) containing 23 per cent (v/v) rabbit serum dialysate. This medium is known to induce a marked mitotic response. In additional studies one lens from each animal was cultured in medium 199 containing 23 per cent (v/v) rabbit serum, and the paired lenses were fixed at identical times. All media contained 200 U. per cubic centimeter of penicillin and were adjusted to an osmolarity of 310 mOsm. per kilogram. This value corresponds to the osmolarity of the rabbit aqueous humor.
The cultured lenses were fixed in a manner identical to the 0 hours preparations. In certain cases, the osmolarity of the fixative was adjusted to either 280, 310, or 322 mOsm. per kilogram. This was accomplished by diluting the buffered fixative with distilled H$_2$O. The rationale for this approach will be presented.

After fixation, the lenses were dehydrated in an ascending series of ethanol followed by propylene oxide. While the lenses were in 100 per cent alcohol, the entire lens was bisected by cutting from the anterior to the posterior pole. Next, a cut was made along the equator of the lens perpendicular to the first cut, which resulted in anterior and posterior quarters. The posterior quarter was discarded and a 1 to 2 mm. wide section was made, passing in the same plane as the initial bisection. Most of the adhering lens fibers were dissected off of the tissue at this time. The result was a continuous narrow section of the anterior portion of the lens which extended from equator to equator through the anterior pole of the lens. A block of tissue from the central region of this section was cut and an additional block of tissue approximately 2 to 3 mm. anterior to the equator of the lens was obtained. These sections are referred to as central and peripheral, respectively. The central and peripheral blocks were further subdivided into 1 mm. blocks and embedded in an Epon-Araldite mixture. Thin sections were stained with uranyl acetate and lead citrate and examined with a Phillips 200 electron microscope.

Results

The descriptions and electron micrographs presented herein represent data obtained from at least 6 lenses for each time period evaluated. Information on lenses cultured in medium 199 containing 23 per cent rabbit serum has been gleaned from tissue fixed at 1, 3, 7, 15, 22, 35, and 48 hours, respectively. Data are also presented on lenses which were cultured in medium 199 or medium 199 containing 23 per cent rabbit serum dialysate and fixed after 1, 3, or 7 hours of culture. For convenience, the results are presented in chronological order. The apical portion of the epithelial cell refers to the region of the cell which is in close proximity to the underlying lens fibers. The basal region of the cell is designated as that portion which is nearest to the overlying lens capsule. Such nomenclature is appropriate for several reasons, including the pattern of embryologic development of the lens and the antigenic similarity between the lens capsule and basement membranes.

**Tissue fixed at 0 hour.** Thin sections obtained from lenses which were fixed immediately after isolation showed the typical orientation of lens capsule, epithelium, and underlying lens fibers. The cells from both the central and peripheral regions of the lens were present, as anticipated, as a monolayer. The prominent features of these sections included numerous intercellular spaces and lateral interdigitations of the plasma membranes. As shown in Figs. 1 and 2, the intercellular spaces, lateral interdigitations of the cell membranes, and pinocytotic vesicles were principally confined to the basal region of the cell subjacent to the overlying lens capsule. The intercellular spaces contained a fine granular material and, in many instances, villi-like projections from adjacent cells protruded into the spaces.

The apical region of the cell, on the other hand, was characterized by the presence of a multilobed nucleus, Golgi apparatus, centrioles, and in many instances by the presence of a relatively narrow band of fibrillar material which was adjacent to the underlying cell membranes. The band of fibrillar material was located between the nucleus and the underlying lens fibers. It was noted at all times evaluated and is particularly evident in Figs. 2 and 7. Moreover, the homogeneous region of fibrillar material exhibited an appearance which was similar to that of the underlying lens fibers. Other cellular structures which were easily recognized in the epithelial cells included mitochondria, endoplasmic reticulum, and several dense bodies which appeared to be lysosomes.

The distribution of these structures demonstrates that the cell is polarized. The intercellular spaces and interdigitations of the cell membranes and pinocytotic vesicles were principally confined to the region of the cell subjacent to the lens capsule, whereas the centrioles, Golgi apparatus, and the homogeneous fibrillar
Fig. 1. Peripheral region of a rabbit lens epithelium which was fixed immediately after isolation (0 hours). Note intercellular spaces (IS) in the basal region of the cell near the overlying lens capsule (Cap). The spaces contain fine granular material and villilike projections from adjacent cells. The apical region, near the lens fibers (LF), is characterized by the presence of the nucleus (N), Golgi apparatus (Go), and hyaline zone (Hz). Free and membrane-bound ribosomes (RER) and filamentous-like material are prominent features of the cells. (×20,000.)
Fig. 2. Peripheral region of a rabbit lens epithelium which was fixed immediately after isolation (0 hours). Note multilobed elongated nucleus (N) with prominent nucleoli (nu). Also note the presence of pinocytic vesicles (pv) in the basal region of the cell immediately subjacent to the lens capsule (Cap). A centriole (c) and prominent hyaline zone (Hz) immediately adjacent to the lens fibers (LF) are characteristics of the apical region. (x25,000.)
region were limited to the apical region of the cell in close proximity to the underlying lens fibers.

It was possible that the presence and/or morphology of the intercellular spaces might be related to the osmolarity of the fixative. To test this, one lens from each animal was placed in fixative which had an osmolarity of 310 mOsm. per kilogram. The contralateral lens was placed in fixative which had an osmolarity of 280 or 322 mOsm. per kilogram. The fine structure of the lenses exposed to these fixatives was evaluated. The results indicated that the intercellular spaces were present at all osmolarities investigated. Moreover, the size and location of the intercellular spaces were approximately the same irrespective of the osmolarity of the fixative.

**Tissue fixed at 1 hour.** The most conspicuous ultrastructural alteration in lenses which were fixed after 1 hour of culture was a change in cell-to-cell relationships in the basal region of the epithelial cells. As is shown in Fig. 3, which is a preparation obtained from a lens which was cultured in medium 199 containing 23 per cent rabbit serum, the basal region of the epithelium was completely devoid of the intercellular spaces which typified the 0 hour preparations of this tissue. In order to see if the change in the intercellular spaces might be related to the composition of the culture media, additional data were obtained from lenses cultured for 1 hour in medium 199 alone or in medium 199 containing 23 per cent rabbit serum. The basal region of the epithelium was completely devoid of the intercellular spaces which typified the 0 hour preparations of this tissue. In order to see if the change in the intercellular spaces might be related to the composition of the culture media, additional data were obtained from lenses cultured for 1 hour in medium 199 alone or in medium 199 containing 23 per cent rabbit serum. The basal region of the epithelium was completely devoid of the intercellular spaces which typified the 0 hour preparations of this tissue. In order to see if the change in the intercellular spaces might be related to the composition of the culture media, additional data were obtained from lenses cultured for 1 hour in medium 199 alone or in medium 199 containing 23 per cent rabbit serum. The basal region of the epithelium was completely devoid of the intercellular spaces which typified the 0 hour preparations of this tissue.

**Tissue fixed at 3 hours.** Electron micrographs of lenses fixed after 3 hours of culture also lacked the intercellular spaces which were a characteristic of 0 hour preparations. In addition, regional ultrastructural differences which consisted of variations in the degree of folding of the cell membrane were first noted at this time. As a case in point, Fig. 4, which is a preparation from the peripheral region of a lens cultured in medium 199 containing 23 per cent rabbit serum, showed extensive and elaborate infoldings of the cell membrane. In the central region of the epithelium (Fig. 5), the foldings of the plasma membrane were decidedly less elaborate. A comparison of Figs. 4 and 5, which represent material obtained from the peripheral and central regions of the same lens, clearly illustrates this regional difference. The changes of the surface membranes were evident in lenses cultured in all three media; however, they were most prominent in lenses cultured in medium 199 containing 23 per cent rabbit serum. In addition, some of the epithelial cells cultured in medium 199 containing 23 per cent rabbit serum dialysate also exhibited an increase in ribosomes. In addition, some of the epithelial cells cultured in medium 199 containing 23 per cent rabbit serum showed very prominent nucleoli. In general, intercellular spaces were not found (Fig. 6). Fig. 7 is the only preparation which exhibited an intercellular space at this time. In addition to these changes, it should be noted that the membranous foldings which were extremely prominent at 3 hours of culture are not as pronounced at 7 hours or, in fact, at any subsequent time which was studied. Fig. 7 also shows the well-developed homogeneous region of fibrillar material which is located in the apical region of the cells.

**Tissue fixed at 7 hours.** The results from lenses cultured for 15 hours and subsequent times represent data obtained from lenses cultured in medium 199 containing 23 per cent rabbit serum. As is shown in Fig. 8, lenses which were fixed at 15 hours of culture showed intercellular spaces similar to those detected in 0 hour control.
Fig. 3. Central region of a rabbit lens epithelium which was fixed after 1 hour of organ culture. Note the absence of intercellular spaces characteristic of lenses fixed at 0 hours. Note the multilobed nucleus (N) with a prominent nucleolus (nu) and Golgi apparatus (Go) located in the apical region of the cell. (×20,000.)
Fig. 4. Peripheral region of a rabbit lens epithelium which was fixed after 3 hours of organ culture. Note the presence of extensive and elaborate infoldings of the cellular membranes (CM) in the basal region of the cell near the overlying lens capsule (Cap). Compare with Fig. 5. (×25,000.)
Fig. 5. Central region of a rabbit lens epithelium which was fixed after 3 hours of organ culture. Note the absence of intercellular spaces and the presence of extensive interdigitating cellular membranes (CM) in the basal region of the cell. Also note the presence of a pinocytotic vesicle (pv). (×15,000.)
Fig. 6. Peripheral zone of a rabbit lens epithelium which was fixed after 7 hours of organ culture. Lenses fixed at 7 hours do not show the prominent intercellular spaces (IS) which are characteristic of 0 hour preparations. The cell membranes in the basal region show extensive interdigitations. (×15,000.)
Fig. 7. Central region of a rabbit lens epithelium which was fixed after 7 hours of organ culture. Micrograph shows an intercellular space (IS) comparable to that found at 0 hours. However, the spaces are relatively rare at this time. There is an apparent increase in the number of free ribosomes. (x20,000.)
Fig. 8. Peripheral region of a rabbit lens epithelium which was fixed after 15 hours of organ culture. Numerous intercellular spaces (IS) which are similar to those found at 0 hours are characteristic of lenses fixed at this time. The epithelial cells are noticeably elongated and the epithelium is multilayered. Cells adjacent to the lens fibers (LF) show convolutions of rough endoplasmic reticulum (RER) and numerous ribosomes. Cells immediately subjacent to the lens capsule (Cap) do not exhibit these features. (×17,500.)
lenses (Fig. 1). The spaces contained a fine granular material which appeared to be similar to the material contained in the intercellular spaces which were noted at 0 hours. The location, size, and content of the intercellular spaces were similar to those found at 0 hours. Moreover, as shown in Fig. 8, the spaces exhibit periodic expansions and, in this respect, are similar to those described by Cohen in the human lens epithelium. In addition to the reappearance of intercellular spaces, cells in the peripheral region of the lens were noticeably elongated, and there was a dramatic change in the over-all organization of the epithelium, including the establishment of multilayering of cells (Fig. 8). The cells that were elongated were rich in ribosomes and lacked the pronounced foldings of the cell membranes. The cells in the central region of the lens, on the other hand, exhibited the typical monolayered pattern which was similar to that noted in Fig. 4. It is of interest that all of the changes noted thus far, i.e., disappearance of the intercellular spaces (1 hour), appearance of extensive foldings of the cell membranes (3 hours), increase in the number of free ribosomes (7 hours), and the reappearance of intercellular spaces coupled with the organizational changes in the peripheral region of the lens (15 hours), precede the initiation of DNA synthesis and mitosis which commence at 22 and 35 hours, respectively.

**Tissue fixed at 22 hours.** As is shown in Fig. 9, the cells from the peripheral region of lenses fixed at 22 hours of culture were more elongated, and multilayering of the epithelium was quite pronounced. Moreover, fibrillar material (Fig. 9) and convolutions of rough endoplasmic reticulum (Fig. 10) were noted in the cells from the peripheral region of the lens and were very prominent in the cells of the multilayer which were in close proximity to the existing lens fibers. The fibrils were arranged in an orderly manner and had an average diameter of 78 Å. The cells of the central epithelium at 22 hours exhibited numerous convolutions of rough endoplasmic reticulum which were absent from this region in lenses which were fixed at 15 hours of culture. The dense fibrous material which was found in cells from the peripheral region of the lens at 22 hours was not evident in the electron micrographs of the central epithelium.

**Tissue fixed at 35 and 48 hours.** At 35 and 48 hours (Figs. 11 and 12), the epithelium was more compact, multilayered, cells and nuclei were extensively elongated, and fibrous material was present. As is shown in Fig. 12, whorls of rough endoplasmic reticulum were very prominent features of these cells. In some instances, cells in close proximity to the lens fibers were very elongated, exhibited numerous ribosomes, showed nuclear degeneration, and contained abundant fibrillar material. Some of these changes may be associated with the differentiation of the epithelial cells into lens fibers.

**Discussion.** Lenses which were isolated and cultured in a medium which is known to induce a marked mitotic stimulation exhibited several fine structural modifications which were not apparent in the 0 hour control preparations. The initial morphologic modifications were confined to the basal portion of the epithelial cells immediately subjacent to the lens capsule. Lenses fixed after 1 hour of culture lacked the characteristic intercellular spaces which were present in lenses fixed immediately upon isolation. As pointed out, these spaces contained a fine granular material, and in many instances villilike projections from adjacent cells protruded into the spaces. Lenses fixed at 1, 3, and 7 hours did not show the characteristic intercellular spaces. It was also shown that by 15 hours of culture intercellular spaces similar in appearance to those noted at 0 hours and similar to those described by Cohen in human lens epithelium were present. The intercellular spaces found at 0 or 15 hours and beyond may be fixation artifacts, normal constituents of the lens, or a response of the lens to isolation and culture. In view of the
Fig. 9. Peripheral region of a rabbit lens epithelium which was fixed after 22 hours of organ culture. This micrograph illustrates the fibrillar material (fib) which is found in many cells at this time. The fibrous material is usually confined to cells of the multilayered epithelium which are located near the lens fibers (LF). (×25,000.)
Fig. 10. Central region of a rabbit lens epithelium which was fixed after 22 hours of organ culture. Numerous convolutions of rough endoplasmic reticulum (RER) are found in cells which are near the lens fibers (LF). This feature is characteristic of cells which are situated immediately adjacent to the lens fibers. The epithelial cells of the central region of the lenses fixed at 15 hours do not exhibit these characteristics. Portions of the epithelium exhibiting multilayering are shown. Also note the presence of thin fibrils (fb) in these cells. (×28,000.)
Fig. 11. Peripheral region of a rabbit lens epithelium which was fixed after 35 hours of organ culture. The cells are extensively elongated, and multilayering is evident. The hyaline zone (Hz) is usually located on the side of the cell near the lens fibers (LF). Intercellular spaces (IS) are prominent in cells near the lens fibers. (×12,000.)
Fig. 12. Peripheral region of a rabbit lens epithelium which was fixed after 48 hours of organ culture. The epithelium is extensively multilayered. Note that whorls of rough endoplasmic reticulum (WRER) which are not found at 22 hours are prominent at this time. (x15,000.)
fact that the spaces are noted at 0 hours and at 15 hours and beyond, with the use of the same fixation procedure as that employed at 1, 3, and 7 hours, a time during which the intercellular spaces are absent, it seems unlikely that they are fixation artifacts. Irrespective of whether the intercellular spaces are normal constituents of the lens or morphologic adaptations to isolation and culture there is clearly a change in tissue organization which is apparent by 1 hour of culture. Current studies on lenses fixed in situ may permit us to distinguish between points 2 and 3. It has been suggested by Wanko and Gavin and Cohen that the intercellular spaces may serve either a nutritive capacity, i.e., as channels which supply the avascular lens with necessary metabolites, or could conceivably represent the morphologic basis for lens accommodation. Our current findings provide no further information about these possibilities.

In an electron microscopic study of galactose-induced cataract in the rat, Kuwabara and associates reported that one of the initial biomicroscopic signs which preceded the appearance of lens cataracts was a widening of the intercellular spaces. The morphology of the intercellular spaces described by these investigators is decidedly different from that observed in the present study. However, the changes in intercellular spaces observed by Kuwabara and associates precede the onset of cell division which is known to accompany sugar cataract. As demonstrated in the present report, such a chronology of events involving changes in tissue organization which precede mitosis is characteristic of the induction of cell division in the cultured lens. It should be noted that the careful studies of Kuwabara and associates do not reveal the presence of pronounced intercellular spaces in the normal rat lens. In addition to the species difference these investigations employed a different isolation and fixation technique from that used in the present study. A comparative study employing various fixation and isolation procedures is quite in order.

It is also of interest that several of the initial changes in tissue organization were first noted in the peripheral region of the lens. As a case in point, the pronounced changes in surface membranes which were noted at 3 hours (Fig. 5) as well as the marked cellular elongation and multilayering which were found at 15 hours (as shown in Fig. 8) were confined to sections obtained from the peripheral region of the lens. At later times, e.g., 35 and 48 hours, pronounced cellular elongation and multilayering were found throughout both the central and peripheral regions of the epithelium. It is of interest that the spatio-temporal pattern of DNA synthesis and mitosis in the cultured rabbit lens follows a similar pattern. That is, cells in DNA synthesis are first noted in the peripheral region of the lens and subsequently throughout the central epithelium. The mitotic response follows a similar pattern. This suggests that there is a relationship between the initial changes in fine structure and the subsequent mitotic response. The changes in intercellular spaces (1 hour), the pronounced infolding of the cellular membranes (3 hours), and initial changes in multilayering and cellular elongation (15 hours) all precede the onset of DNA synthesis.

In addition to the changes in tissue organization, an increment in the number of free ribosomes was noted at 7 hours of culture. Moreover, an increase in the incorporation of tritiated uridine and tritiated protein hydrolysate is known to occur in the epithelial cells of the cultured rabbit lens. It should be noted that the increase in ribosomes was apparent in lenses cultured in medium 199 containing either 23 per cent rabbit serum or 23 per cent rabbit serum dialysate. It seems likely that the increase in ribosomes at 7 hours represents an adjustment of the lens to the process of isolation and culture. This interpretation is compatible with the work of Bagchi, who demonstrated an increase in the incorporation of tritiated uridine in rabbit lens epithelium cultured in a variety of culture media, i.e., medium 199, medium 199
containing 23 per cent rabbit serum, or 23 per cent rabbit serum dialysate. The increased uptake of tritiated uridine was noted at 7 hours of culture.

The morphologic changes noted in the current study are in broad agreement with light microscopic studies on the injured lens under in vivo conditions. In both the frog7 and rabbit lens,4,8 mechanical injury evokes a sequel of events including cellular elongation, migration, and augmented macromolecular synthesis, all of which precede cell division. Cogan42 has pointed out that the mere wrinkling of the lens capsule can evoke a mitotic response. Studies at the ultrastructural level on mechanically injured rat lens indicate marked changes in association between neighboring cells.20 Gierthy, Bobrow, and Rothstein43 have devised a technique which permits the direct observation of lens epithelial cells maintained in organ culture. These investigators noted pronounced changes in cell-to-cell relationships prior to mitosis. An attractive hypothesis which incorporates these observations is that the initial event which triggers cell division and/or releases the cell from the contact inhibition of growth44 may be a physical alteration in cell-to-cell relationships. It is possible, for example, that changes in tissue organization may be indicative of physiologic changes such as “uncoupling” of the cells, which has been proposed as an important factor in the control of cell division.15,45 If this is true, the initial trigger of cell division may be a modification of the cell surface. The possible role of changes in the cell surface in the regulation of cell division has been emphasized in a number of recent studies.36, 46 Needless to say, at some point there must be a correlation between the physical changes in the cell surface and a derepression of genes which regulate cell division.

REFERENCES