to be $\mathcal{H} - \mu N$. Transition to a situation where the time dependence is given by the true Hamiltonian $\mathcal{H}$ can be accomplished by means of a gauge transformation, and consideration of the effect of this transformation on the electron operators gives immediately Gor'kov's result $F \propto \exp(-2\,i\,\mu\,t/\hbar)$.
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Cortical Control of Cell Division

The cell surface appears to determine some specific events of division in *Stentor* and egg cells.

Noël de Terra

Recently, evidence has accumulated in support of the hypothesis that the surface membrane of animal cells plays an important part in the regulation of cell division. Much of this evidence has come from work on three systems: the ciliate *Stentor*, egg cells, and mammalian cells in culture. When the results obtained from work on these three cell types are considered together, they complement each other in a very interesting way. The work on *Stentor* has provided direct experimental evidence that cell surface changes are involved in timing the cell cycle, regulating some major events of organelle replication, and effecting cytokinesis; it has not yet yielded information about the biochemical nature of these changes. By contrast, the work on cultured cells has shown the existence of specific biochemical surface changes associated with progress through the cell cycle (*1*) or with neoplastic transformation (*2*), but has not demonstrated that these changes are involved in regulating any specific events of cell division. The work on egg cells serves as a bridge connecting the work on *Stentor* with that on cultured cells because it has suggested that the cell surface regulates some specific events of cell division in mitotically dividing cells as well as in the amitotic divisions of ciliates. In this article I review the work on *Stentor* and egg cells and show how the data from these systems complement and reinforce the conclusions arising from work on cultured cells. A broader view of the problem is thus obtained by examining it simultaneously from these different vantage points.

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Cortical Control in Stentor

The interphase cell. Stentor coeruleus (Fig. 1) is a trumpet-shaped ciliate which can extend to a length of about 1 millimeter. Its large size and exceptional powers of wound healing have made it a favorable experimental organism for work involving cell microsurgery. The anteriorly located oral apparatus contains a band of oral membranelles (fused plates of cilia originating from rows of basal bodies). This band encloses a circle of cortex (the frontal field) which spirals into a gullet. About 100 rows of blue-green pigment granules run longitudinally down the body. These are graded in width and the widest and narrowest stripes meet on the ventral surface to form the "locus of stripe contrast" (*3*). The pigmented stripes alternate with clear stripes containing the somatic kineties (ciliary rows); these are longitudinal rows of paired basal bodies from which originate cilia and various fibrillar structures. The subcortically located chain macronucleus spirals almost the entire length of the cell. About 40 to 60 tiny diploid micronuclei are scattered along the macronuclear chain.

The cortex of *Stentor* consists of (i) a surface membrane continuous over both the cell body and the ciliary axonemes, and (ii) various structures situated beneath this membrane to a depth of 3 to 5 micrometers. Most prominent among these are the kinetosomes together with the various microtubule systems originating from them (that is, ciliary axonemes, K_m fibers) and the microfilamentous M-bands or myonemes.

Cell division in Stentor. Most major events of cell division in *Stentor* are events of organelle replication. Indeed, the first sign of division (Fig. 2) is the assembly of basal bodies at the locus of stripe contrast; these form the oral primordium which gives rise to the feeding structures of the posterior daughter cell. These newly formed basal bodies sprout cilia and align themselves in rows to form the oral membranelles. The developing membranellar band lengthens and curves, and the posterior end then invaginates to form a gullet. The oral apparatus migrates upward to its final position as the cleavage furrow separates the two daughter cells.

During division, the chain macronucleus undergoes a sequence of morphological transformations (coalescence, elongation, nodulation). These changes double the number of macronuclear nodes, thus preserving the nuclear chain which is presumably advantageous in terms of increased surface area. The diploid micronuclei undergo mitosis while these macronuclear changes are taking place.

Induction of cell division by structural changes in the cortex. Because the first sign of incipient cell division is the assembly of basal bodies that form the oral primordium, one might expect the endogenous stimulus for initiating oral primordium formation to be extremely important in understanding the mechanism for initiating cell division. Tartar (*3*) has, in fact, shown that cell division can be induced prematurely by operations which induce oral primordium developments. *Stentor* regenerates a new oral apparatus if all or part of the original feeding structures are microsurgically removed. If this operation is performed on cells which look large enough to be nearing division (predivision cells), the induced oral primordium formation which follows is accompanied by cleavage, and the stentor divides prematurely. By contrast, small stentors which are not normally capable of cleavage replace their microsurgically reduced oral structures with new ones but do not form a cleavage furrow and divide. These findings strongly suggest that cell division may normally be initiated by whatever endogenous conditions initiate oral development in large cells that are not dividing. The question of what these endogenous conditions are thus becomes of paramount importance in understanding the mechanism for initiation of cell division. Oral primordium formation occurs during cell division and regeneration (Fig. 3); it can also occur spontaneously during interphase to replace the intact feeding structures with larger ones in a process called reorganization (Fig. 4). One can thus approach the problem of what stimulus initiates oral development by comparing these three processes and asking what feature might be common to all of them.

The minimal stimulus to regeneration is known; this process can be induced microsurgically by decreasing the size of the oral apparatus relative to the size of the cell body (*3*). It is therefore extremely interesting that a similar reduction in the relative size of the oral structures develops gradually during the cell division cycle because the oral apparatus does not grow during interphase but the cell body does (Fig. 5). This fact suggests that oral primordium formation during cell division occurs at some specific, "critical" oral/somatic ratio when the oral apparatus becomes disproportionately small in relation to the size of the cell body. This hypothesis can also explain spontaneous replacement of oral structures with larger ones during interphase (reorganization). The significance of this process has long been obscure. Reorganization, like regeneration, is of interest here because it provides important insights into the nature of the endogenous stimulus for oral development which also seems to be the endogenous stimulus for cell division.

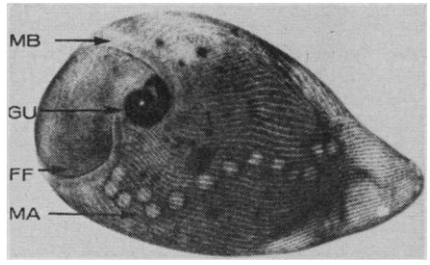

Fig. 1. *Stentor* flattened in microcompression chamber. *MA*, macronucleus; *MB*, membranellar band; *GU*, gullet; *FF*, frontal field. [Courtesy of Academic Press]

Reorganizing stentors may be cells that have attained the critical oral/somatic ratio postulated as the general stimulus for oral primordium formation while they are still too small to be capable of cleavage. This could occur whenever fluctuations in food supply result in periods of starvation followed by periods of growth. During starvation, both the oral apparatus and the cell body become progressively smaller and a miniaturized stentor results. During subsequent resumption of feeding and growth, only the cell body enlarges, because the oral apparatus cannot increase in size except through primordium formation. Growth of a previously starved cell would therefore eventually produce a stentor with disproportionately small oral structures and a cell body below the minimum size required for cleavage, and one might therefore expect oral development to occur without fission. If this hypothesis is correct, it should be possible to produce reorganization experimentally by miniaturizing stentors through starvation or microsurgery and then allowing them to grow. This prediction has been verified experimentally (*4*; Fig. 6). The hypothesis also predicts the existence of a specific, critical oral/somatic ratio associated with primordium formation during division and reorganization. This prediction has also been verified through measurements showing that a specific, characteristic ratio between the surface area of the oral apparatus and that of the cell body (0.05 to 0.06) exists during the early stages of both division and reorganization (*4*). Regeneration after excision of oral structures presumably represents the extreme case where the oral/somatic ratio is reduced below the critical value to zero.

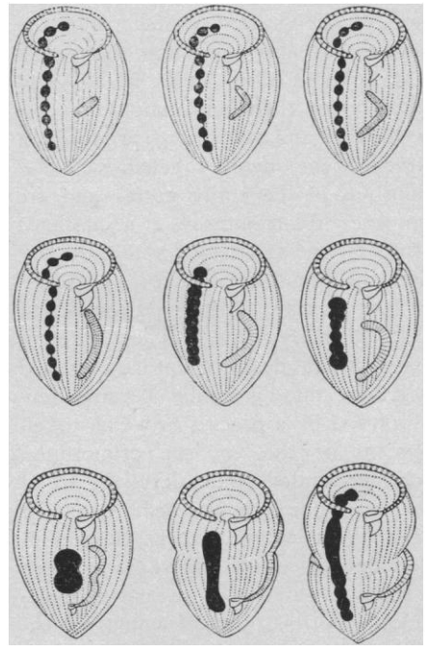

Fig. 2. Stages of cell division in *Stentor*. Top row, left to right: stages 1, 2, and 3, showing oral primordium as stippled structure located posterior to the gullet. Middle row, left to right: stages 4, 5 (early), and 5 (late); oral primordium is now curved with rows of basal bodies aligned to form membranelles. Bottom row, left to right: stages 6, 7, and 8; gullet is formed at posterior end of oral primordium. [Courtesy of Cambridge University Press]

The experiments I have described suggest that the stimulus for oral differentiation (and therefore for cell division) is a specific configuration of the cell surface which develops gradually during interphase as a consequence of cell surface growth.

Two experiments indicate further that it is, specifically, a change in cell surface structure (rather than cell volume or mass) which initiates oral development. The first is Tartar's demonstration that primordium formation can be induced microsurgically by simply rotating the oral apparatus 180° in situ (*3*). While this operation obviously produces a change in the structural organization of the rigid cell surface, it would not be expected to affect the organization of the fluid endoplasm. The second experiment bearing on this point involves the behavior of dividing

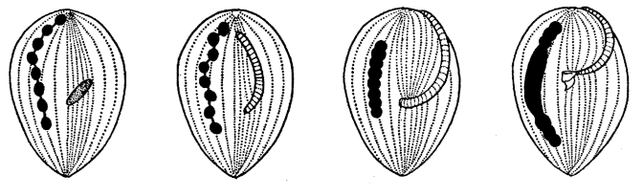

Fig. 3 (left). Some stages of regeneration in *Stentor* showing formation and development of oral primordium. Left to right: stages 2, 4, 5, and 8. [Courtesy of Cambridge University Press]

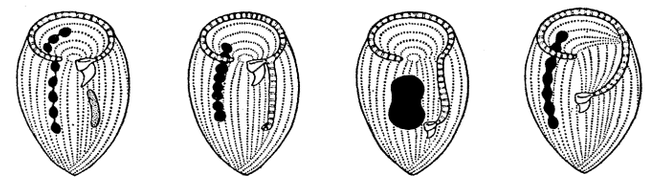

Fig. 4 (right). Some stages of reorganization in *Stentor*, showing resorption of original gullet and its replacement by a new one associated with the developing oral primordium. Left to right: stages 2, 5, 6, and 8. [Courtesy of Cambridge University Press]

cells from which the developing oral primordium has been excised. These cells often fail to complete cleavage because of mechanical damage to the presumptive furrow line. They consequently emerge from this abortive division with the same size relationships found in cells prior to division, except that the visible surface structures have become disconnected at the furrow line to form two distinct cortical stripe systems which remain separate for several hours. An oral primordium promptly appears at the posterior locus of stripe contrast (Fig. 7); although the organism has a set of intact oral structures, the fact that these have been "disconnected" and somehow isolated by structural changes in the cell surface during cleavage presumably induces primordium formation in the posterior cortical stripe system. This result therefore also suggests that induction of oral differentiation depends on the occurrence of structural changes in the cell surface (*4*).

In summary, the experiments on *Stentor* have suggested that the endogenous stimulus for oral development and cell division is the presence of a specific pattern or configuration of the cell surface. This pattern develops gradually during interphase as a consequence of cell surface growth and can be identified because in ciliates much of the structural organization of the cell surface is visible. These results are not surprising, since work on other ciliates has provided abundant evidence that the structural organization of the cortex determines both the time and place at which various cortical organelles will develop (*3, 5*).

Cortical control of organelle replication. The first indications that the cortex provides some factor required for basal body assembly and consequent oral primordium formation came from experimental studies by Tartar (*6*). He found that when cell sectors bearing early primordia (stages 1 to 3) were transplanted heteropolar (rotated 180°) into regenerating cells at the same stage of development, the grafted primordia generally failed to develop but were resorbed instead. Sometimes only the grafted primordium was resorbed while the host primordium continued to develop; sometimes both primordia were resorbed, in which case the host primordium reappeared later and developed although a primordium did not appear in the grafted sector. By contrast, if cell segments bearing early primordia were grafted to regenerating stentors in homopolar orientation they always developed normally. Since the heteropolar primordia which failed to develop could presumably receive through the endoplasm any diffusible substances required for regeneration, Tartar concluded that these could not be the only factors required for primordium development but that structural integration of the grafted segment into the cortex was also important. He wrote (*3*): "These cases may be of great interest for their implication that the instigation and support of primordium development involves geometric relationships in the entire cortex and are not solely the result, say, of a substance like RNA being released within the cell and affecting formative loci regardless of how they lie." Tartar (*6*) also found that when two stentors in different stages of oral development were grafted together, they generally became synchronized by a process in which development of the younger primordium was accelerated while development of the older one was retarded. Again, he drew the conclusion that primordium development must involve more than a successive release of regulatory molecules since this by itself would not explain why the older primordium was retarded. He therefore suggested that primordium development was likely to involve an "organizational message in the cortex" in addition to diffusible substances in the endoplasm.

Further evidence for cortical control of oral primordium formation comes from my experiments (*7*). When half of a regenerating stentor is grafted heteropolar to half of a morphostatic (nondifferentiating) cell, the former usually induces primordium formation in the latter within 3 to 4 hours (*3*). The graft boundary is at first marked by a transparent line which disappears as the granule-studded and visibly more opaque cortical membranes of the graft components are drawn together and become continuous. This transparent line presumably represents a precipitation membrane formed during the operation, since the cell contents are visible beneath but do not escape. When the two graft components are separated by a thin disk taken from a third cell (Fig. 8), two visible lines of discontinuity are present, but healing now occurs more slowly than in the simple heteropolar grafts as judged by visual observation and the tendency of graft complexes flattened under coverslips at various times after the operation to break open at the graft boundaries. When a central disk is present, the number of graft complexes showing primordium induction in the morphostatic component declines from 68 percent to 18 to 19 percent. Experiments in which the food vacuoles of one graft component were labeled with polyvinyltoluene spheres before the operation showed that the labeled vacuoles began passing across the graft boundary immediately after the operation; the two members of the graft complex therefore demonstrably shared a common endoplasm. These results strongly suggest that the stimulus inducing primordium formation in the morphostatic component travels across the cell surface rather than through the endoplasm and affects the locus of stripe contrast by interaction with it over a very short distance rather than by diffusion through the endoplasm. Since induction of primordium formation can occur under conditions where the fibrillar structures of the two graft components remain separate [heteropolar doublets (*3*); graft complexes with a middle barrier ring (*7*)], the induction

stimulus is probably transmitted via the striped cortical membrane, although transmission might also occur by way of some cortical entity that has not yet been revealed by electron microscopy.

The morphological events of macronuclear replication (coalescence, elongation, and nodulation; Fig. 2) are most easily studied in regenerating cells because regeneration can be synchronously induced in large numbers of cells by exposing them briefly to 10 percent sucrose, which causes them to shed the membranellar band. When half of an interphase stentor is grafted heteropolar to half of a cell in stage 3 of regeneration, the two members of the graft complex always become synchronized with respect to macronuclear morphology. The rationale behind the following experiments was exactly the same as for the experiments on cortical control of primordium formation; a thin barrier disk from a third cell was placed between the two graft components to create slow-healing cortical discontinuities so that it would be possible to determine whether the synchronizing stimulus travels through the endoplasm or through the cortex (Fig. 8). In these graft complexes, the striped cortical membrane is discontinuous at the graft boundaries after the operation and heals gradually within 3 to 4 hours. Experiments in which the food vacuoles of one graft component were labeled with polyvinyltoluene spheres showed that the two members of the graft complex shared a common endoplasm after the operation. It was found that the presence of cortical discontinuities reduced the induction of nuclear synchrony from 100 percent to 0 percent, suggesting that the induction stimulus travels across the cell surface rather than through the endoplasm and acts at close range on the subcortically located macronucleus (*7*).

The occurrence of these macronuclear changes is dependent on the time of cell division (*3*, *8*), which in turn appears to be set by changes in the configuration of the cell surface during interphase (*4*). If the cortex is timing the cell cycle, it seems reasonable that it should also be responsible for controlling replication of organelles such as the macronucleus and basal bodies which are normally kept "in step" with the cell cycle in such a way that they are not lost or reproduced too frequently.

After an initial growth (G1) period of 1 to 8 hours, macronuclear

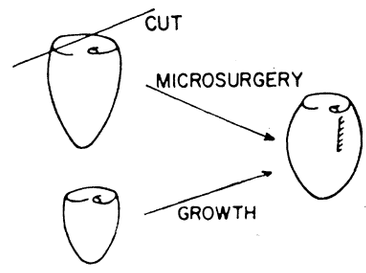

Fig. 5. Growth during interphase and microsurgical reduction of oral structures results in a similar cortical pattern and in oral differentiation.

DNA synthesis in *Stentor* is continuous throughout interphase (36 to 48 hours) and stops during division at the time of macronuclear coalescence (stage 6) (*9*). Macronuclear coalescence also occurs during regeneration induced by removing the membranellar band, which is a specific part of the cortical fibrillar system. If macronuclear DNA synthesis stops at stage 6 during regeneration as well as during division, it would suggest that termination of DNA synthesis can be controlled, at least indirectly, by structural changes in the cortex. This was tested by selecting dividing cells from well-fed cultures; the membranellar bands were removed from half of the daughter cells (group 1) while the other half (group 2) served as controls. When macronuclear coalescence had occurred in the cells of group 1, both groups were transferred to culture medium containing [H^3]thymidine, then prepared for autoradiography. Examination of the autoradiographs showed that the control cells were all engaged in DNA synthesis; by contrast, no label was present above the coalesced nuclei of the regenerating cells. Appropriate controls ruled out the possibility that the absence of macronuclear DNA synthesis in group 1 cells was caused by the sucrose treatment or by their inability to take up [H^3]thymidine (*10*). These observations indicate that termination of DNA synthesis can indeed occur as a consequence of structural changes in the cortex.

Cortical control of cleavage. Tartar (*11*) found that patches of cortex containing part of the presumptive furrow line will divide at the appropriate time (stage 7) if they are transferred from their normal position to other parts of the cell during division (stages 2 to 6). By contrast, the grafted patch will not divide if it is taken from above or below the presumptive furrow line or from a morphostatic cell. Some event therefore occurs during interphase or early division which prepares one part of the cortex, the presumptive furrow line, for fission, perhaps by causing structural changes within it. The dependence of fission on cortical structure is suggested by yet another experiment: if a cortical patch containing the presumptive furrow line is rotated by 90 degrees on a large cell before the onset of division, the cleavage furrow cannot pass through it.

Tartar (*11*) has also shown that furrow formation in grafted patches is not autonomous but depends on infor-

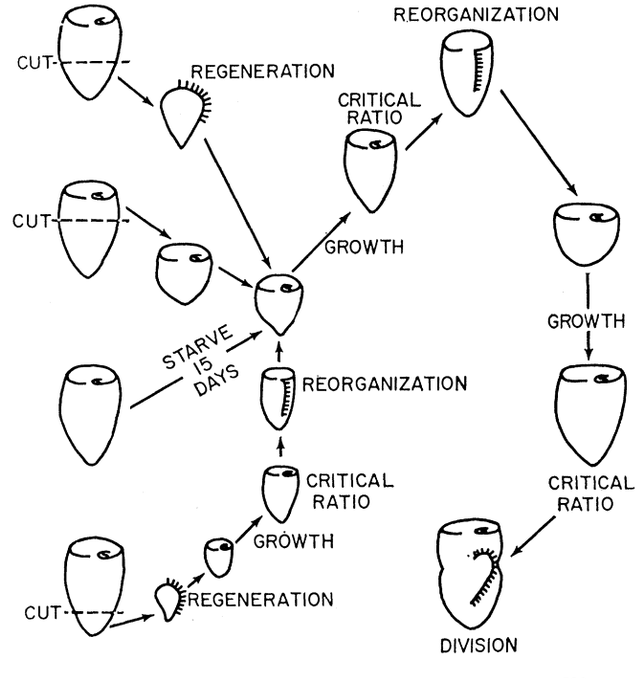

Fig. 6. Summary of experiments showing how reorganization can be induced experimentally by miniaturizing cells in different ways and allowing them to grow. See text for details. [Courtesy of Academic Press]

mation coming from outside the patch. If the host cell is at a later stage of division than the transplanted cortical patch, the patch accelerates and undergoes fission at the same time as the host. If the host is at a later stage of division than the patch and completes division shortly after the graft is made, the patch will not divide. Patches of competent cortex from cells in early division stages will divide if transferred to dividing cells but not to interphase cells or to cells undergoing reorganization. These results suggest that a specific region of cortex "prepared" for division beforehand is activated at stage 6 by a stimulus that induces furrow formation. This stimulus must occur throughout the cell (or cell surface) since grafted patches can cleave when transplanted to abnormal positions.

There is some evidence that the position of the cleavage furrow, as well as its time of appearance, is determined by overall cortical pattern; if shedding of the membranellar band is induced during early division, the furrow is displaced upward (*3*).

These experimental results strongly suggest that the mechanism determining the position of the cleavage furrow, as well as the mechanism for division itself, is located in the cortex. The fibrillar structures of the clear stripes do not seem to be involved, since formation and deployment of the furrow line are not affected by major irregularities in the stripe pattern provided that the striping remains longitudinal (*3, 11*). It is possible that the regulatory mechanisms for control of fission depend on properties of the cortical membrane. In the ciliate *Nassula*, a ring of contractile microfilaments is present just beneath the pellicle in association with the cleavage furrow (*12*); the results obtained by Tartar on the control of cleavage in *Stentor* suggest the presence of a similar mechanism in this ciliate. Work on factors controlling microfilament formation in the terminal web of salamander gut epithelial cells has suggested that the cell membrane may be capable of determining the appearance of microfilaments directly beneath it (*13*). A reasonable hypothesis for regulation of cleavage in *Stentor* is that a specific region of the cell surface becomes structurally modified during late interphase or early division in such a way that it can respond to a later stimulus by causing the appearance of microfilaments beneath it.

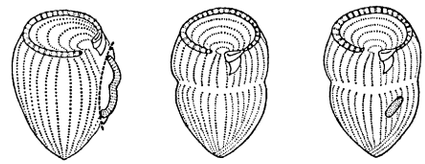

Fig. 7. Role of the cortical fibrillar system in regulating oral differentiation during cell division. (a) Oral primordium excised from stage 5 dividing cell; (b) cleavage fails but kineties disconnect at the furrow line; (c) 4 to 6 hours later, an oral primordium appears at the lower locus of stripe contrast. Oral primordia shown as stippled structures located posterior to gullet. [Courtesy of Cambridge University Press]

In summary, the work on *Stentor* has suggested that: (i) Changes in the structural organization of the cell surface initiate the process of cell division; (ii) during cell division, the cell surface passes through a series of changes which may control some events of organelle replication; and (iii) these regulatory changes do not seem to be caused by release of diffusible substances into the endoplasm.

In the following section I will discuss some work on eggs that indicates that the same principles may operate in at least some mitotically dividing cells.

Cortical Control in Egg Cells

Induction of cell division by structural changes in the cortex. The mechanism for regulation of cell division in eggs is somewhat specialized because these cells do not grow in size between divisions and, consequently, they become smaller and smaller as development proceeds. Obviously, the mechanism for timing the cell cycle in eggs cannot involve cell surface growth in the same way that it appears to in *Stentor*. Nevertheless, it is a surface change (the cortical reaction) brought about by sperm penetration that releases the egg from its quiescent state and sets in motion the sequence of events that culminate in the first cell division. The sperm must interact with the cell surface, for sperm injected into the egg do not initiate development (*14*). In some marine invertebrate eggs, the cortical reaction is visible as a wave of color change sweeping over the egg surface; Runnström (*15*) ascribed this to a changed orientation of lipid molecules in the cortex. In any case, there is widespread agreement that it represents a change in the structural organization of the egg surface. Observation of surface changes during fertilization led some workers to compare the cortical reaction with nerve conduction (*16*) and to suggest that it involves a "chain reaction" or a propagated conversion of microscopic subunits in the cortex (*17*). The discovery of allosteric interactions between the subunits of enzymes has provided a possible explanation for propagated waves of structural change at the molecular level; Changeux and his co-workers (*18*) have extended these principles to membrane structure and suggested that the fertilization reaction, as well as nerve conduction, may involve the conversion of membrane subunits or "protomers" from one conformation to another.

There is no direct evidence for cortical control over the timing of the egg division cycle as there is in *Stentor*. However, cleavage rate does appear to be determined by the cytoplasm rather than the nucleus in at least one instance. Sand dollar eggs and sea urchin eggs have very different cleavage rates. Moore (*19*) found that when nonnucleated and nucleated egg fragments from *Dendraster* are fertilized with *Strongylocentrotus* sperm, they cleave at the same rate as control whole *Dendraster* eggs also fertilized with *Strongylocentrotus* sperm, and this rate is characteristic of *Dendraster* rather than *Strongylocentrotus*. These data are at least consistent with the hypothesis that the timing mechanism is located in the cortex.

Cortical control of organelle replication. In eggs, as in ciliates, there is some reason to think that specific events of organelle replication may be controlled by the cell surface although the evidence is scarcer and more inferential in eggs because the cortex lacks visible signs of polarity and cannot be so conveniently manipulated.

1) During the maturation of *Xenopus* eggs, breakdown of the germinal vesicle, which releases the egg from meiotic prophase, can be induced by externally applied progesterone; the hormone has no effect when injected into the cell interior (*19*). This suggests that progesterone exerts its effect by interacting directly with the egg surface. After germinal vesicle breakdown, development proceeds to metaphase of the second meiotic division from which the egg can be released by

insemination or by pricking it with a glass needle; both stimuli affect the egg surface. Masui and Markert (*20*) found that germinal vesicle breakdown could be induced by injection of cytoplasm from progesterone-treated eggs even when these eggs had been enucleated before exposure to the hormone. Centrifugation experiments showed that the cytoplasmic factor inducing germinal vesicle breakdown was predominantly localized in the hyaline ooplasm which contains the endoplasmic reticulum and a structureless ground substance (*20*). This result is of great interest in view of the suggestion by Jacob and his co-workers (*21*) that the cell surface might affect the nuclear membrane of eukaryotes by transmission of stimuli through the endoplasmic reticulum connecting them.

Further evidence for cortical control of organelle replication in *Xenopus* eggs comes from the work of Brachet and Hubert (*22*). They showed that mitotic abnormalities such as multipolar mitoses, lagging chromosomes, and chromosome bridges occurred in eggs with slight cortical injuries. The occurrence of abnormalities in centriole replication suggests that this process may be cell-surface-dependent in eggs as it is in *Stentor*. The chromosome abnormalities suggest that some event involved in replication of the nucleus may also be controlled by the cell surface, although it is not clear at the present time what process is specifically affected.

2) Work on marine invertebrate eggs also suggests that centriole assembly and pronuclear migration may be controlled by the cell surface. Some of the evidence comes from observations on partially fertilized sea urchin eggs. These are eggs in which the fertilization reaction has been arrested in its progress by a brief heat treatment or by immobilizing the eggs in glass capillaries (*23*) so that the cortex is only partially converted to the fertilized state. The unfertilized portion of the egg retains its cortical granules and does not show the color change which occurs in the cortex of some species during fertilization. The unfertilized part of the egg can be fertilized later and therefore has not been damaged. The sperm pronucleus cannot migrate into the unfertilized portion of the egg; instead, it stops at the boundary between fertilized and unfertilized cytoplasm where it undergoes several aberrant divisions. Runnström (*24*) has written: "The evidence points to an effect on the centrosome. . . . The astrospheres which reflect the centrosome activity may sometimes disappear rather suddenly leaving an uncleaved enlarged nucleus or the first division may be completed but thereafter the division apparatus may vanish." On the basis of these observations, Runnström *et al.* (*25*) concluded: "As the centrosome has to react with the cortex during the penetration of the spermatozoon, its replication also seems to be dependent on an interaction with the cortical layer." How could interactions occur between the centrioles (centrosomes) and the cell surface? The centrioles are connected to the cell surface through the asters and it seems possible that centriole-cortex interactions might take place through this connection. One possible mechanism is suggested by a hypothesis recently proposed to explain why the tail fibers of T4 phage cannot attach to the base plate in the absence of the head. King (*26*) suggested that the structure of the tail is modified by attachment of the head; the resulting conformational rearrangements might then be transmitted from the tail to the base plate, modifying its structure so as to provide sites for tail fiber attachment. In a similar way, some formed structure present in the asters (such as microtubules) could transmit structural rearrangements from the cell surface to the centrioles, changing their conformation in such a way as to create nucleation sites for the production of new centrioles. Microtubules are known to exist in at least two states of structural organization (*27*).

The fact that migration of the male pronucleus is often impaired in partially fertilized eggs suggests that the centrioles, which are brought in by the sperm, may be affected in another of their functions: their ability to serve as microtubule organizing centers. Chambers and Chambers (*28*) concluded from micromanipulation studies that migration of the sperm pronucleus is caused by growth of the sperm aster. They wrote: "The sperm monaster plays a major role in bringing about the union of the male and female pronuclei. Following insemination of the ovum, the growing sperm monaster, an expanding gelated body which de-

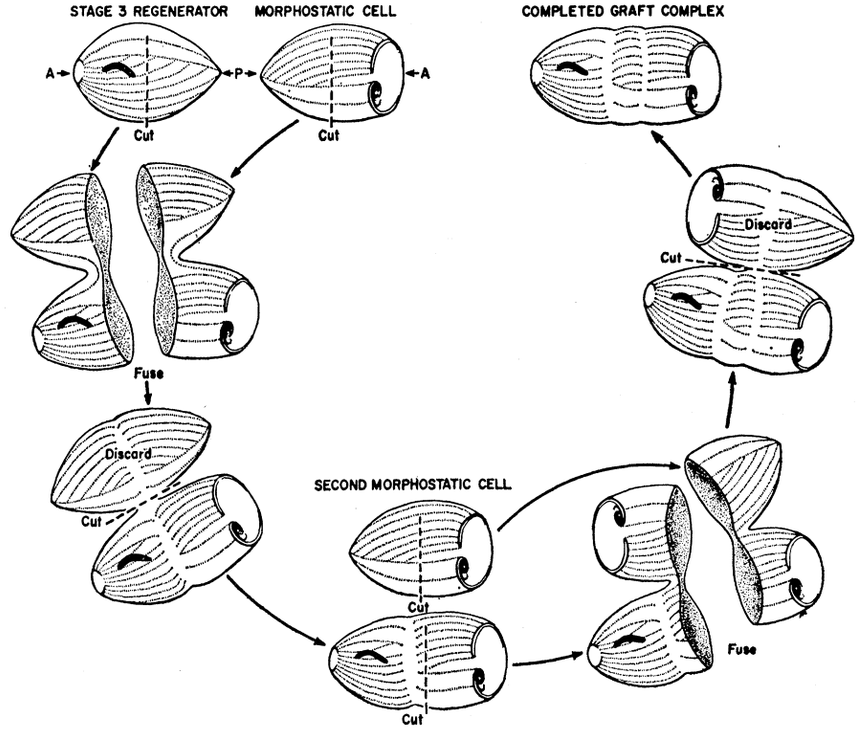

Fig. 8. Procedure used in making graft complexes. The anterior half of a cell in stage 3 of regeneration is grafted to a morphostatic stentor in heteropolar orientation. A cut is then made in the morphostatic stentor just below the line of heal and the anterior half of a second morphostatic cell is added in homopolar orientation with respect to the first one. Most of the first morphostatic cell is removed during the operation so that it persists only as a thin disk separating the two main graft components. *A*, anterior; *P*, posterior. [Courtesy of Academic Press]

velops around the neckpiece of the existing spermatozoon, carries the sperm pronucleus from its initial position just under the egg surface to the center of the egg." Colchicine blocks pronuclear migration in echinoderm eggs (*29*), which suggests that growth of the astral microtubules terminating near the sperm centriole provides the force that carries the sperm centriole and the male pronucleus associated with it into the center of the egg (*30*). There is thus some reason to think that microtubule assembly as well as centriole replication cannot proceed normally in partially fertilized eggs; since centriole production involves microtubule assembly, it seems likely that these two abnormalities result from disruption of the same function. Allen and Hagström (*23*) concluded from the differential behavior of organelles located beneath the fertilized and unfertilized portions of partially fertilized eggs that the mechanism controlling pronuclear migration was regulated by a "nondiffusible" factor, and the hypothesis of centriole-cortex interaction through the formed components of the aster could explain the results which led them to advance this hypothesis.

Finally, certain changes in the properties of the cortex (that is, birefringence and viscosity) are associated with specific stages of the division cycle in eggs; this finding is at least consistent with the hypothesis that cell surface changes determine some events of organelle replication (*31*).

Cortical control of cleavage. In marine invertebrate egg cells, as in *Stentor*, microsurgical studies have suggested that furrow formation is closely associated with events occurring at the cell surface (*32*). The cleavage mechanism must be located in the cell surface, because the nucleus, the mitotic spindle, and the subcortical cytoplasm can all be removed without cleavage being affected [for review, see (*32*)]. Microsurgical studies, in which the geometrical relationships between the mitotic apparatus and the cell surface are altered in various ways, have suggested that the position of the furrow is determined by the mitotic apparatus, specifically by the concerted action of the asters on the cell surface. Some of these experiments resulted in the simultaneous formation of multiple furrows separated by only a few micrometers, leading Rappaport (*32*) to think that furrow formation was not likely to be controlled by release of a diffusible substance. He suggested that the cell surface might be modified by "infection of the surface with some propagable change in molecular structure" and later found that the modifying stimulus travels at the rate of microtubule growth (*33*), which is somewhat slower than the rate one would expect for a diffusible substance. Cleavage might therefore involve a structural change propagated from the asters to the cell surface, just as control of centriole behavior might involve a structural change propagated from the cell surface to the centrioles through the asters. The phenomenon of multiple furrow formation is strongly reminiscent of certain events in ciliate morphogenesis; during reorganization in *Stentor*, for instance, the gullet is resorbed while a new one is developing near it and it is equally difficult to explain this by a mechanism involving diffusible substances released into the endoplasm; the basis is more likely to be structural.

In summary, work on marine invertebrate eggs has indicated that the cell surface may regulate the time of cell division, centriole replication, and cleavage; this work has repeatedly led investigators to invoke the concepts of nondiffusible factors and propagated structural change even before any satisfactory theory was available to explain such phenomena at the molecular level.

Extensions and Connections

The work described in the previous sections has suggested that in both *Stentor* and egg cells cortical changes may control the time of cell division and that the cortex may be involved in determining certain events in the replication of the nucleus and centrioles or basal bodies (centrioles bearing cilia). The work on these cell types has further indicated that some of these phenomena cannot be adequately explained by mechanisms involving release of diffusible substances into the endoplasm.

Why should these conclusions have emerged, specifically, from work on these particular cell types? Most obviously, because these cells are large enough to permit the kind of microsurgical experiments which have, in general, led to such ideas. However, there may also be a second, important reason: both ciliates and eggs share a requirement for a rigid cortical layer. Ciliates appear to depend on a rigid surface layer both to maintain the positions of the major surface organelles concerned with feeding and locomotion and to distribute these properly during cell division; eggs require a rigid surface layer because they apparently use the cortex to parcel out regulatory substances involved in bringing about tissue-specific protein differentiations (*34*). There may be some basic difference between the surface structure of eggs and ciliates, compared to mammalian cells which seem to have a more fluid surface layer (*35*). The basis for this apparent dissimilarity could be the presence or absence of rigid supporting elements in the cortical layer; it might also be caused by some intrinsic difference in membrane structure. The two main membrane constituents, lipids and proteins, could exist either as lipids distributed within a protein matrix or proteins distributed within a lipid matrix, and these two types of membrane structure could specify very different physical properties (*36*).

In spite of the apparent differences in surface properties between ciliates and egg cells compared to cultured cells, there is increasing evidence that the cell surface plays a major role in controlling the divisions of mammalian cells. Some of this evidence has come from work showing differences in the surfaces of cultured cells after transformation with oncogenic viruses which are thought to interfere with normal mechanisms controlling growth and cell division (*2*, *37*). Other studies have demonstrated the existence of specific surface changes during mitosis or during different stages of the growth cycle (*1*). A third line of evidence derives from work showing that the interactions of various substances with cell surfaces stimulate cell division, for example, plant lectins (*38*), proteases (*39*), and tumor-promoting agents (*40*). There is as yet no evidence for control of organelle replication by the cell surface in cultured cells. However, it is very interesting that tumor cells, which are known to have abnormal surfaces, so often show mitotic abnormalities of the type found by Brachet and Hubert (*22*) in *Xenopus* eggs after slight cortical damage (that is, multipolar mitoses, lagging chromosomes, and chromosome bridges) (*41*). Aberrant centriole positioning has also been observed in tumor cells (*42*). The work on *Stentor* and egg cells strongly suggests that mitotic abnormalities of

organelle replication in tumor cells will prove to be a consequence of abnormal surface structure.

Summary

Experimental work on the ciliate *Stentor* has provided direct experimental evidence for the hypothesis that the cell surface controls the time of cell division and also plays a part in determining the replication of the macronucleus and basal bodies during division. Experimental studies on amphibian and marine invertebrate eggs have led to similar conclusions and therefore provide a bridge between the work on *Stentor* and the work suggesting control of cell division by the cell surface in the mitotic divisions of mammalian cells. The observations on egg cells and *Stentor* have further suggested that it may prove necessary to invoke mechanisms involving propagated structural change rather than diffusion to explain at least some of these phenomena.

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43. This research was supported by grant GB-24544 from the National Science Foundation, institutional grants CA-06927 and RR-05539 from the National Institutes of Health, and an appropriation from the Commonwealth of Pennsylvania.

Educational Challenges for the University

Significant educational progress and innovation require superior talent and a changed university role.

F. Reif

The present educational role of the university seems incongruous with its expected role of intellectual leadership. Indeed, one might naively expect that the university would regard its educational function as crucially important since it is the only institution entrusted with high-level educational tasks (while carrying on research functions somewhat similar to some of those pursued by other institutions, such as the National Institutes of Health or Bell Telephone Laboratories). One might suppose that the educational function of the university would have assumed even greater importance in recent years because of the enormous growth of knowledge and the large increase in the number of people demanding to be educated. Finally, one might expect that the university would be a spearhead in educational innovation since it has played such a successful innovative role in most other areas (such as the sciences or technology). Interest in educational innovation might be presumed to be particularly high since progress in this area would have a direct bearing on the university's own mode of functioning. Furthermore, there has been thoughtful discussion about the great potential of a prospective "educational revolution." For example, the Carnegie Commission on Higher Education recently published a report suggesting a blueprint for the implementation of such a revolution (*1*).

These expectations are in marked contrast to current realities. In actuality, the university is largely preoccupied with the maintenance of standard educational programs and seems content to formulate educational policy in terms of mundane criteria such as degree requirements, the faculty-student ratio, or the number of class-contact hours. Except for some graduate education closely connected with the research of the faculty, the university's norm in the area of education is reasonable adequacy, rather than excellence or innovative leadership. The university does not systemati-

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