

Simulation Testing of Mechanisms for Inducing the Formation of the Contractile Ring in Cytokinesis

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Abstract. There is persuasive evidence that the role of the mitotic apparatus (MA) in cytokinesis is to control the location of the cleavage furrow. The geometric aspects of this interaction between the MA and the cortex are complex and, thus, computer simulation can be a useful means for testing hypotheses about the induction process. White and Borisy (1983. *J. Theor. Biol.* 101:289–316) used computer simulations to show that long-range signals from the asters, varying inversely as various powers of distance, produce summed effects that are minima at the equator of spherical cells. Their results have seemed to support the “polar relaxation” class of hypotheses, in which the effect of the asters is to weaken cortical contractility so that contraction becomes maximized at the equator because it is least inhibited there.

However, the experimental studies of Rappaport and Rappaport (1988. *J. Exp. Zool.* 247:92–98) indicate that the asters actually strengthen cortical contractility.

In this paper, we use computer simulation to determine how signals from the MA will need to vary in effect as functions of distance to cause cortical contractility to become maximized where the furrows are to be induced. Although we confirm that inverse power inhibitory signals could induce equatorial furrows in spherical cells, we also find that this ability is destroyed by flattening, constricting, or distorting cells into cylinders, geometries for which Rappaport's experiments show furrows form (1986. *Int. Rev. Cytol.* 105:245–281). We then show that stimulatory signals of the right kind would induce furrows at the locations observed, in spherical cells as well as cells distorted by experimental manipulation. These signals must be constant out to a threshold distance but decrease abruptly beyond that distance. We also show that this ability depends on having the “drop-off” threshold occur at just the right distance relative to the dimensions of the cell and separation of the asters.

DESPITE convincing evidence that the physical cause of cleavage in animal cells is the contraction of a cortical ring of acto-myosin (Kiehart et al., 1982; Schroeder, 1973) and clear indications that the mitotic apparatus (MA)¹ induces the formation of this cortical ring (Rappaport, 1988), the mechanism of this induction remains unknown. Because cleavage has been shown to continue once initiated, even after the MA has been removed, we can apparently be certain that the MA is not itself the mechanical cause of mitosis (Yatsu, 1912; Hiramoto, 1956). Other experiments, in which the MA is moved before cleavage (Rappaport, 1985), show conclusively that the MA somehow signals to the cortex where to form the cleavage furrow.

The most obvious candidate for the source of this furrow-inducing signal might seem to be the central part of the MA around the metaphase plate simply because the contractile ring forms directly over this region in the most familiar cases of cleavage. There are, however, many cases in which furrows are also induced midway between the asters of two different MAs, even though these asters are not connected by a spindle, implying that the actual sources of the furrow-

inducing signals are the asters. For example, in Rappaport's (1961) classic experiments with cells squeezed into the shape of a torus, furrows formed midway between the asters of one MA and another. Comparable events are part of the normal development of many species that pass through multinucleated stages of development. The coelenterate *Renilla* is one example (Wilson, 1903), although a much more extreme case occurs during the blastoderm stage of development of insects, such as *Drosophila*, when thousands of intersecting cleavage furrows form simultaneously as a hexagonal network, converting the egg from a syncytial to a cellular state (Warn et al., 1984; Lundquist and Lowkvist, 1984). The purpose of this paper is to explore alternative mechanisms by which aster signals could induce maximum concentrations of cortical contractility halfway between one aster and another.

Mechanisms capable of this result can be divided into two basic classes: one involving inhibition of contractility and the other involving its stimulation. The first possibility is that the signal from the asters weakens cortical contractility, but that this weakening is least at the site overlying the spindle equator. The second possibility is that the signal from the asters stimulates or strengthens cortical contractility and

1. Abbreviation used in this paper: MA, mitotic apparatus.

the resulting strengthening is maximal at the site overlying the spindle equator. These alternatives prove remarkably difficult to distinguish experimentally, considering that they are essentially opposites.

There is experimental evidence for an overall increase in cortical contraction just before mitosis followed by a decrease at the time of cleavage: cells tend to round up at the onset of mitosis and there is a change in the resistance of their surfaces to mechanical deformation (Schroeder, 1981). But, although this increased contraction has been taken as support for the first of the two possible mechanisms above, it is actually consistent with either. Both hypotheses depend upon the induction by the asters of an unbalanced distribution of cortical contractility which, in turn, causes a mechanical redistribution and realignment of the contractile proteins toward whichever part of the cell surface has the greater contractility, either because it has been least inhibited or because it has been most stimulated. Thus, a direct test between the two hypotheses requires some method for mapping the relative magnitudes of tensile stress at different parts of the cell surface; but no such direct method is yet available. The best alternative has been to impose changes in cell shape (and/or the positions and spacing of the asters), thus altering the relative distances over which the signals are required to act; one can then observe whether (and where) the cleavage furrow forms. Many ingenious shape-distorting experiments of this kind have been carried out by Rappaport (1986), who has determined which changes in cell geometry do (or do not) prevent the formation of cleavage furrows.

Unfortunately, it can be very difficult to relate the results of such shape-changing experiments to the two alternative mechanisms for inducing cleavage furrows. This is because the distortions of cell geometry produce so many simultaneous changes in distances between the asters and the different parts of the cortex. Difficulties of this kind can probably best be overcome by means of computer simulation. For any hypothesis regarding the mode of the asters' effects on the cortex and for any given alteration in cell geometry, computers can calculate and map the patterns of relative cortical contractility. The predictions of any theory can then be displayed graphically and compared with experimental observations.

Such computer simulations were used by White and Borisy (1983) to analyze several aspects of animal cell cleavage. Their study began with simulations that mapped the expected spatial distributions of contractility in the cortex of cells with spherical, ellipsoidal, and certain other shapes; they also simulated the relationship between cell surface curvatures and the spatial distribution of cortical contractility as well as the realignment of contractile elements in response to tension gradients. Their contractility mapping was based on the assumption that a signal emanates from the center of each aster and that the effect of this signal decreases in strength according to some mathematical function of distance between the asters and the cortex. The mathematical functions that they used were the inverse square and inverse fourth power of distance. They found that, when the expected effects of the two functions were summed at each point on a spherical cell surface, such inverse power functions would form a minimum in a band running around the cell surface at its equator.

Because White and Borisy found no mathematical function of distance that would cause the aster signals to sum to a maximum at the equator, their results have seemed to support

the "polar relaxation" mechanism for the induction of the cleavage furrow (i.e., the first possibility listed above). On the other hand, their simulations considered the effects of only a very limited range of mathematical functions and cell shapes. In particular, White and Borisy did not consider the various altered cell geometries that Rappaport had produced experimentally to ask whether inhibitory signals decreasing with inverse powers of distance would correctly predict whether cells with such distorted geometries could still produce cleavage furrows. This is what we have tried to do.

Materials and Methods

We have written a series of simple computer programs to calculate spatial distributions of effects of signals passing from the asters to the cell cortex depending on (a) the cell shape, (b) the aster positions, and (c) the mathematical function or curve by which the effects of the aster signals vary with the distance separating each aster from the different parts of the cell surface. These programs were written in the Pascal language and run on Macintosh SE and Macintosh II computers (Apple Computers, Inc., Cupertino, CA); most programs were also written in the C language and Suncore and run on a SUN III computer (Sun Microsystems, Inc., Mountain View, CA). Both sets of programs map the summed effects of the asters on the cell cortex and they do so (a) for any arbitrary mathematical function of distance (including even hand-drawn functions in the case of the Pascal programs), (b) for any arbitrary cell shape (even for hand-drawn shapes), and (c) for any choice of position or spacing of the asters within the cell. Printed copies of these programs will be provided to any interested reader who requests them.

The relative dimensions (i.e., aster separation distance relative to cell diameter) for the spherical cell used for the initial sets of calculations were based on data provided to us by Prof. Raymond Rappaport, based upon his measurements of normal eggs of the sand dollar *Echinarchnius parma* at the one-celled stage. Their aster separation distance is 36% of their diameter.

Our methods for generating and testing the consequences of arbitrary cell shapes and mathematical functions made use of arrays to store series of discrete values. To create the semicircular outlines, for example, one uses the following instructions: for $n = 0$ to $MAXN$ do begin $x[n] := XCENTER + RADIUS * \cos(n * \pi / MAXN + \pi)$; $y[n] := YCENTER + RADIUS * \sin(n * \pi / MAXN + \pi)$; end; To flatten the equatorial region, the y -coordinate values were reassigned by the following: for $n = 0$ to $MAXN$ do begin if $y[n] > limit$ then $y := limit$; end (for progressively decreasing numerical values of the "limit" variable). To use the computer mouse to change the cell shape, we used the angle subtended by the current mouse position relative to the position of the cell center, thus controlling which particular point along the cell periphery is currently being reassigned: e.g., $subtend := \pi - \arctan((XCENTER - mouseLoc.v) / (mouseLoc.h - YCENTER))$; $i := \text{round}(subtend * MAXN / \pi)$; $x[i] := mouseLoc.h$; $y[i] := mouseLoc.v$;

Note that this method of cell outline generation cannot produce certain concave shapes. This discrete description of the surface makes it easy to determine the distances between each aster and a point on the outline. The distances are used to determine the net signal at the point via the signal function of distance. This simple method of surface signal determination does not take into account surface concavities that block the effect emanating from one of the asters at a surface point (i.e., block the aster's line of sight to the surface). Such cell shapes are beyond the scope of this paper but deserve consideration both experimentally and in future simulations; this issue is related to the question of whether aster effects can act around corners.

The use of the mouse to create arbitrary functions of distance is more straightforward: e.g., $i := mouseLoc.h + XOFFSET$; $stimulus[i] := mouseLoc.v + YOFFSET$; (the offsets adjust for position of mouse input on the screen). In this way, one creates what amounts to a fine-grained step function, with 200 steps in the versions used here. The sigmoidal functions were generated by intercalating part of a sine wave between two assigned limits: e.g., for $i := 0$ to LR do $stimulus[i] := 0.5$; for $i := LR$ to UR do $stimulus[i] := stimulus[0] - stimulus[0] * (\sin(\pi * (i - LR) / (UR - LR) - \pi / 2) + 1) / 2$; for $i := UR$ to $MAXDIST$ do $stimulus[i] := 0$; (with LR and UR being, respectively, the distances at which the function begins and ends its decrease).

The inverse power functions for noninteger variable exponents were

generated by the following: function affect (dist:real):real; begin; affect := $\exp(n \cdot \ln(\text{dist}))$; end.

The representation of variables as apparent three-dimensional height (as that in Fig. 7) was achieved by plotting the y component above its usual position by an amount proportional to "effect" and also by adding a fraction of the displacement in the y direction to the value of the x position: e.g., `moveto(round(xtest+ytest/2), round(ytest+effect))`.

Results

Comparison with Earlier Findings by White and Borisy

Initially, we follow our predecessors' simplifying assumption that the aster effects vary only as functions of distance and are additive. Later, we consider how the results would be affected by other variables (such as barriers, including other asters): such temporary assumptions are merely a means of keeping issues separate from one another, by considering variables one at a time.

When we use our programs to map the distribution of signal over the surface of a spherical cell, letting signal magnitude vary inversely with the square of the distance between the aster center and each particular part of the cell surface, we confirm the findings of White and Borisy (1983) (Fig. 1). The summed effects of the signals from the asters become a minimum along the cell equator. Thus, if the effect of these signals were a weakening of cortical contractility, the result would be a concentration of contractility along the cell equator, explaining furrow induction there. Notice, however, that with this form of distance dependence the minimum is quite shallow and broad, with the equatorial region being inhibited 59% as much as the poles for the inverse square function.

When the inhibitory effects vary inversely with other powers of distance between asters and cortex, the resulting spatial distributions of relative effects are found to be qualitatively similar to those produced by the inverse square function (Fig. 2). Equatorial minima were produced by all inverse powers from 1 to 10 (including noninteger powers at intervals of 0.1). These minima become progressively deeper with increasing powers, and the bottoms of the minima become broader and flatter with the larger exponents. Nevertheless, based on the assumption that furrows would form where the inhibition of cortical contractility was least, equatorial furrows would be induced (in spherical cells at least) by signals whose inhibition of contractility vary as any inverse power of distance.

The Effects of Equatorial Flattening, as in Cylindrical Cells

The computer programs were then used to determine the behavior to be expected when signal magnitudes obey inverse power laws in nonspherical cells. In view of Rappaport's many observations of cleavage in echinoderm eggs that had been reshaped into cylinders (for example see Rappaport 1981), we paid special attention to the redistribution of aster effects when a spherical cell was progressively constricted equatorially, thus becoming more and more cylindrical. The sequence of cross-sectional shapes is shown in Fig. 3 together with plots of the resulting redistributions of the effects of the aster signals. This particular plot is based on an inverse square variation of aster signal magnitude as a function of distance; but the results are qualitatively the same for all other inverse powers.

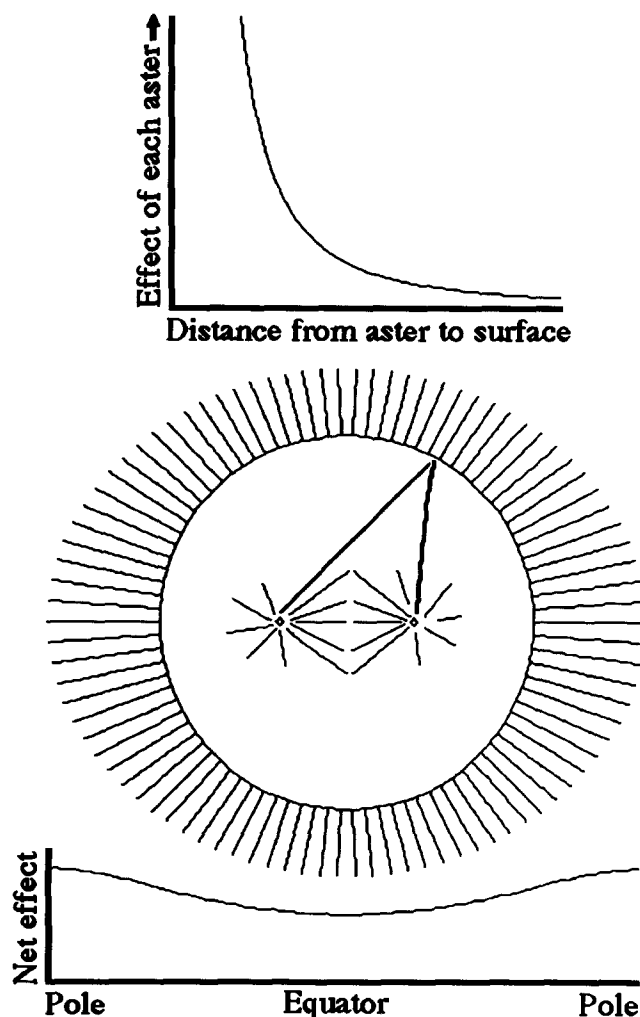


Figure 1. Net signal from the two asters at different points around the spherical cell periphery for the case in which signal strength decreases as the inverse square of the distance from each aster. The graph on the top shows the inverse square function to scale. The cell outline is shown in the middle, including the positions of the two asters. The length of each line radiating from the surface is proportional to the net effect at that part of the cell surface. The relative amounts of effect are also graphed (as height) at the bottom, with the x-axis representing half of the cell's circumference (from one pole to the other, with the equator at the middle).

It is apparent in this figure that even very slight equatorial flattening causes the equatorial minimum to be split in two by the induction of a small maximum at its center. This means that an inhibitory signal, varying as an inverse power of distance, would no longer produce a single equatorial contractile ring (once the equator has been constricted even slightly). Instead, these results imply that two rings would form, one on either side of the equator (corresponding to the two new inhibition minima). As the degree of equatorial constriction is increased, these local minima move farther apart; they are located at the edges of the area of flattening. The new central maximum grows progressively higher with more flattening until, eventually, it is itself split into two peaks with a new (third) minimum between them. This would mean that, if a cell were constricted into a narrow enough cylinder,

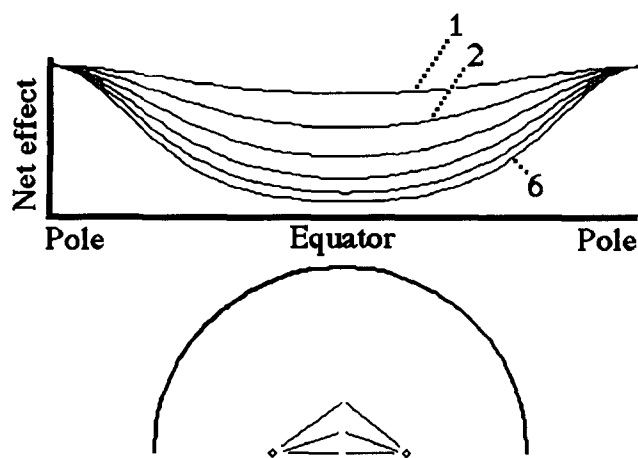


Figure 2. (Top) Relative amount of net aster signal at each point of the spherical cell periphery which would result for effects varying as each of the first six inverse powers of distance; the topmost curve graphs the distribution of net effect for the inverse first power, the next line below it corresponds to the inverse square function, etc. (Bottom) The cell profile and aster positions are shown.

it might be expected to recover its ability to form an equatorial furrow.

These same results are found when the effects of the asters are made to vary inversely with other powers of distance; inverse powers from 1 to 10 were tried in steps of 0.1 (Fig. 4 shows the integral values from one to six for 20% flattening). For all of these inverse powers even slight equatorial constriction destroys the central minimum by splitting it into two for small degrees of flattening and into three when flattening is further increased.

We also carried out analogous studies of the effects of equatorial constrictions in which the cell surface is actually indented rather than merely flattened. Such experimentally

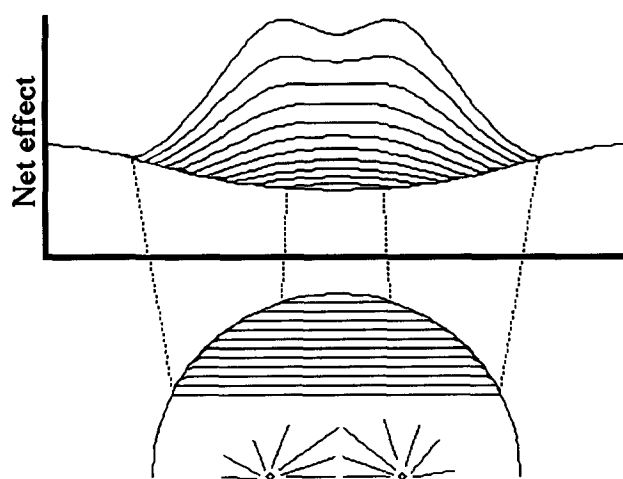


Figure 3. Distributions of net aster signal which would occur when a spherical cell is compressed equatorially into a series of cylindrical shapes. The graph at the top shows relative amounts of net effect around the cell periphery for cells constricted by amounts increasing from 0 to 55% in 5% increments. The lowest curve of the graph corresponds to the unconstricted cell, while the topmost (bimodal) curve shows the distribution resulting from the largest constriction. The cell outlines and aster positions are shown at the bottom.

imposed furrows in the cell surface have been shown by Rappaport and Rappaport (1988) not to block the subsequent induction of the normal cleavage furrow. The results of our simulations of these experiments were equivalent to the results of constriction into cylindrical shapes, as discussed above. Even the smallest amounts of imposed constriction result in the splitting of the previous equatorial minimum into two. The results were the same for all inverse power functions and again show that the results of Rappaport's experiments are contrary to the predictions of a polar inhibition model based on signals whose effects diminish with any inverse power of distance.

In the simulations shown in Figs. 3 and 4, the length of the constricted cell has not been increased to maintain constant volume as would be expected. However, we carried out computations in which this elongation was included and found that the splitting of the equatorial minimum was even more pronounced. The extra minima were also sharper and required less equatorial constriction to form. This additional element was not included in the figures to avoid confusing the two issues.

On the other hand, when our simulations were applied to the situation of cells reshaped into prolate ellipsoids, our results exactly confirmed White and Borisy (1983; cf. their Fig. 11): i.e., the equatorial minimum is not destroyed, although new minima are also produced at either pole. When cell shapes were changed progressively through a series of increasingly elliptical shapes, the resulting distribution of effects remained qualitatively the same, with a single equatorial and two polar minima, and there was no intermediate elliptical shape in which the equatorial minimum was destroyed or split. These results indicate that the ability of inverse power functions to produce equatorial minima depends on having the equatorial part of the cell surface curve out away from the axis of the MA.

Testing Alternative Patterns of Signaling

Because no inverse power function was able to predict correctly how furrow induction would be affected by equatorial constriction, we looked for alternative mathematical func-

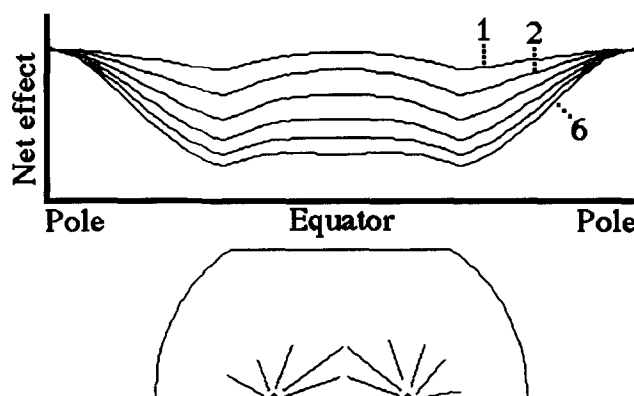


Figure 4. Distributions of net aster signal around the periphery of a slightly flattened or constricted cell (of the shape diagrammed at the bottom) when the aster effects are made to vary with distance according to the first six inverse power laws. The topmost curve is for the inverse first power, the curve below it for the inverse square, etc.

tions able to induce furrows in not only spherical but also flattened, cylindrical, or constricted cells. The capacity of our programs to test the consequences even of hand-drawn curves allowed us to test a wide variety of such functions. These tests brought to light some general rules. In particular, we find that the ability of inhibitory signals to induce furrows in flattened and constricted cells is improved by making the inhibition vary with distance according to a sigmoidal curve in which the magnitude of the signal is relatively constant out to a certain threshold distance but then drops off rapidly as the distance becomes larger than the threshold. (This threshold distance needs to be slightly less than the shortest distance from the aster centers to the part of the cortex where the furrow is to be induced.)

The more sharply the curve drops off and the flatter the plateaus are above and below this drop-off, the greater is the ability of the mechanism to tolerate flattening and constriction of the cell surface (while still achieving the induction of an equatorial minimum of cortical inhibition and, thus, the induction of a furrow). Of course, there is a limit to what can be accomplished in this way: no curve can drop off sharply enough to induce cleavage in a cell whose equator has been squeezed into an artificial furrow so deep that its lowest parts are already closer to the asters than is the rest of the cell surface.

Therefore, we also used these same computer programs to calculate and map the distribution of relative effects that would be produced at different parts of the cell surface if the effects of the aster signal were to stimulate or strengthen cortical contractility rather than to inhibit or weaken it. Of course, in the case of stimulatory functions, the prediction is that the contractile rings and cleavage furrows should be induced to form along those loci where the signal effects are maximal (instead of along the minima, as was the case when considering inhibitory signals).

Although we have tried a wide variety of differently shaped curves, we find that the best match to actual cell behavior is achieved by a sigmoidal curve like that shown in Fig. 5 (and very much like the sigmoidal curve just discussed above in relation to possible inhibitory signals). This is because the induction of a contractility maximum along the equator of a spherical cell requires that the aster effects overlap maximally at the equator, but as little as possible elsewhere (just as Rappaport had previously deduced). This means that the signal magnitude should be maximal out to this threshold distance (which therefore needs to be slightly greater than the distance from each aster to the equator) but needs to drop off sharply at larger distances. In other words, the stimulation effects need to extend to (or just beyond) the furrow site. It should not be much weaker there than at shorter distances or extend very far beyond the locus of furrow induction (lest the whole cell cortex be stimulated equally). In the case of a spherical cell, these requirements are illustrated in Fig. 5 which shows the sigmoidal shape needed by the mathematical curve (cf. Fig. 1 in Rappaport, 1969).

When a curve of this shape is made to govern the relative amounts of stimulatory effects produced at various distances from the asters, then maxima of contractility (and thus contractile rings) will be induced in the equatorial plane bisecting the MA, and this result will not be prevented by flattening or constricting the cell surface (as is shown in Fig. 6). Thus, cylindrical cells will still be able to cleave. Notice the con-

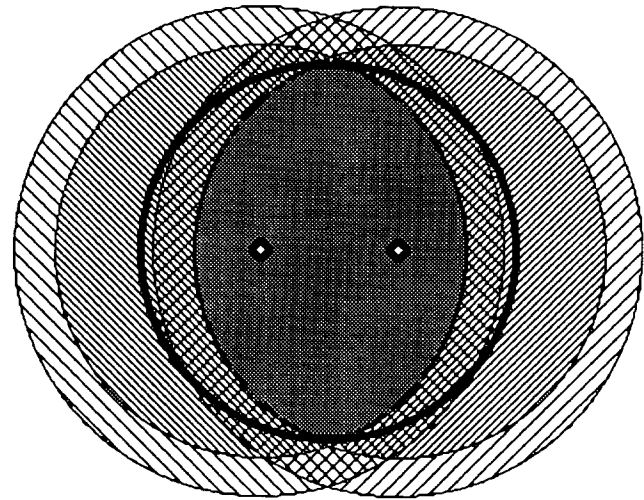
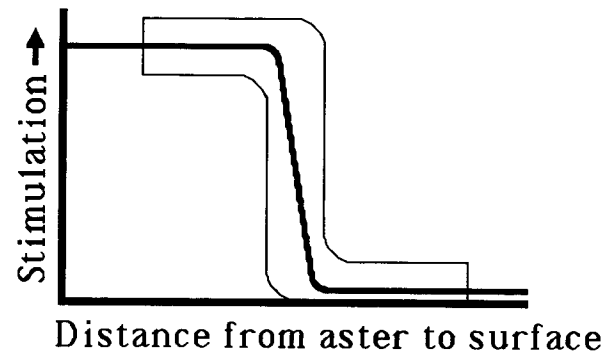


Figure 5. Graph of the function of aster-cortex distance by which the aster signal needs to vary for equatorial maxima of stimulation to be produced not only in spherical but also in flattened and constricted cells. The diagram at the bottom shows the loci over which the aster signals need to decrease with distance, superimposed on a circular cell shape (see text for fuller description).

trast between these results and those described above for inverse power functions: this time, the equatorial maximum is not destroyed or split by the distortion of cell shape. In fact, this maximum is even somewhat accentuated by the constriction.

Using this same sigmoidal function, we have also modeled the expected spatial distribution of net stimulation that would occur when any asters are distributed in a plane beneath the cell surface (for example, in the blastoderm stage of a *Drosophila* embryo; Fig. 7). In this pseudo three-dimensional representation, apparent height is used to represent the relative amounts of aster stimulation at each location on the two-dimensional surface: i.e., the apparent peaks and ridges of this diagram represent loci of maximum stimulation. Notice that these maxima become distributed as an approximately hexagonal network. The areas at the centers of each hexagon (which are flat in the diagram) correspond to the areas of the cell surface directly over the asters: the asters were assigned positions in a plane below the cell surface at a depth that was made equal to 90% of the spacing distance between adjacent asters. This hexagonal pattern matches that actually formed by the cleavage furrows of the *Drosophila* blastoderm, as well as the hexagonal network of actin concentrations which

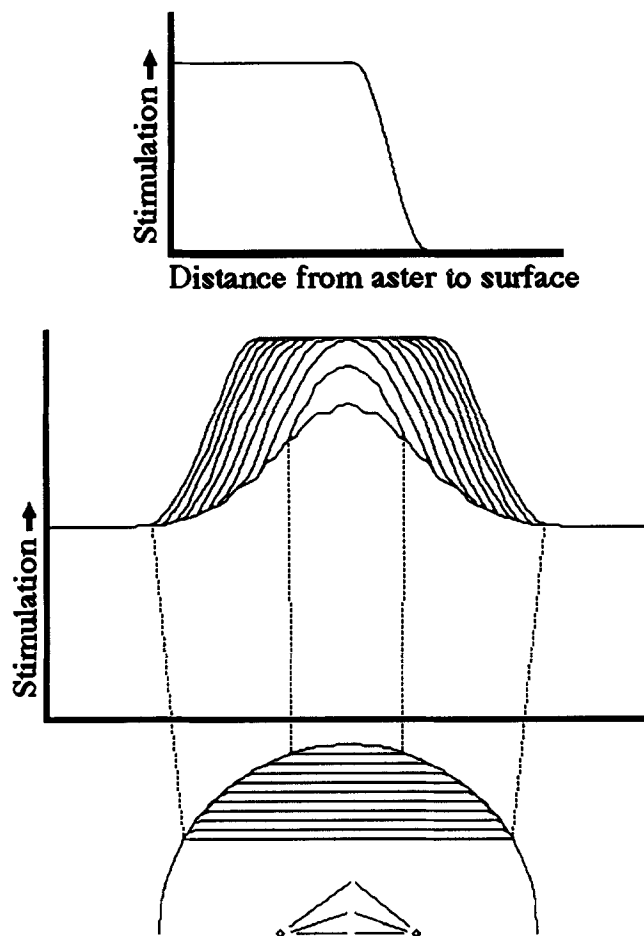


Figure 6. Distributions of net aster signal along the cell surface resulting from signals that vary with distance according to the sigmoidal curve shown at the top. The graph in the middle shows distributions of net effects when the cell shape is flattened or constricted by amounts increasing from 0 to 50% in 5% increments. The lowermost line of the graph corresponds to no constriction, while the uppermost line corresponds to 50% constriction. The different degrees of flattening are diagrammed at the bottom.

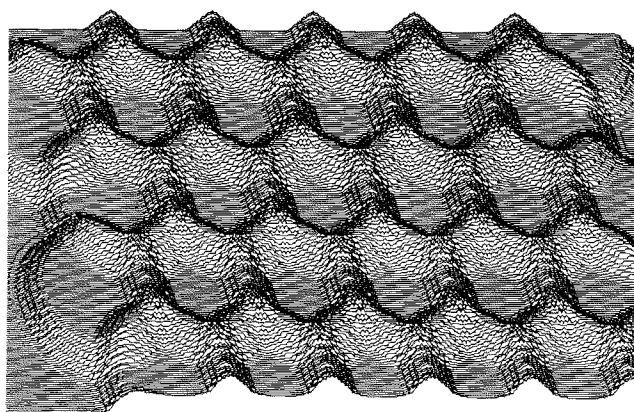


Figure 7. Relative amounts of net aster signal on a flat surface beneath which many asters are distributed in a planar layer below the cell surface. The amount of stimulus is graphed as apparent height above the surface for each point and forms a hexagonal network of maxima. This pattern resembles the hexagonal network of actin and myosin that accumulates in the *Drosophila* egg cortex before its simultaneous cleavage into several thousand cells.

have been observed to presage these furrows (Warn et al., 1984). This same program was also run using the inverse square function and other inverse powers of distance and resulted in nearly homogeneous distributions of summed signal over the cell surface.

The Critical Dependence of Stimulation on the Range of the Effect

The computer results reveal an important weakness of sigmoidal functions as explanations for furrow induction. Their ability to produce maxima at the correct location turns out to be strongly dependent on having the drop-off occur within exactly the right range of distances. Furthermore, this narrow range of distances was itself found to vary, depending on cell dimensions. These dimensions include the diameter of spherical cells, the distances between one aster and the other, and (in the case of the *Drosophila* situation) the depth of the asters beneath the cell surface. Fig. 8 shows that for the case of a spherical cell there are major differences in the distribution of net signal at the cell surface as a result of progressively increasing the range of the aster signals.

For a spherical cell, with diameter, D , in which the two asters are separated by the distance, L , the successful induction of an equatorial contractility maximum will depend on the aster signal magnitude being relatively constant out to a distance of approximately the square root of $D^2/4 + L^2/4$ and then dropping off precipitously between there and a distance of $D/2 + L/2$. When this drop-off occurs at too great a distance, which is equivalent to the cell being too small relative to the range of the aster signals, then no maximum is produced. Conversely, if the drop-off occurs at too close a range, equivalent to the cell being too large, then the result is actually an equatorial minimum instead of a maximum. To yield equatorial furrows, a stimulatory effect would need to decrease with distance according to a mathematical function

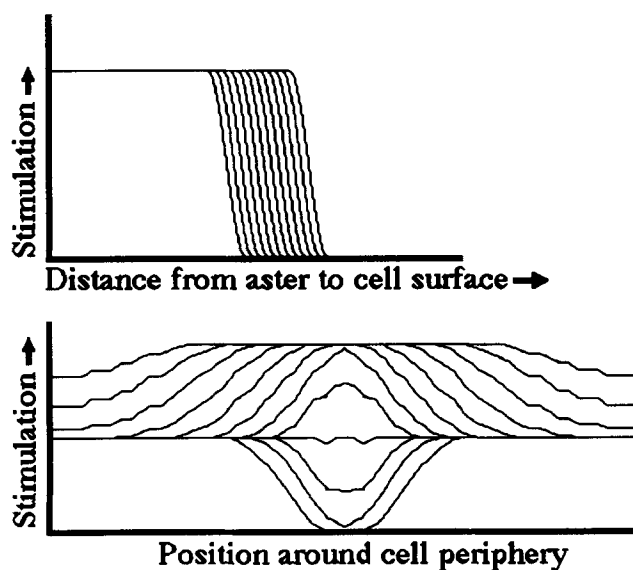


Figure 8. Relative amounts of net aster signal at the surface of a spherical cell depending on the distance at which aster effect decreases sharply. The lowermost curve of the bottom graph corresponds to the leftmost curve of the upper graph.

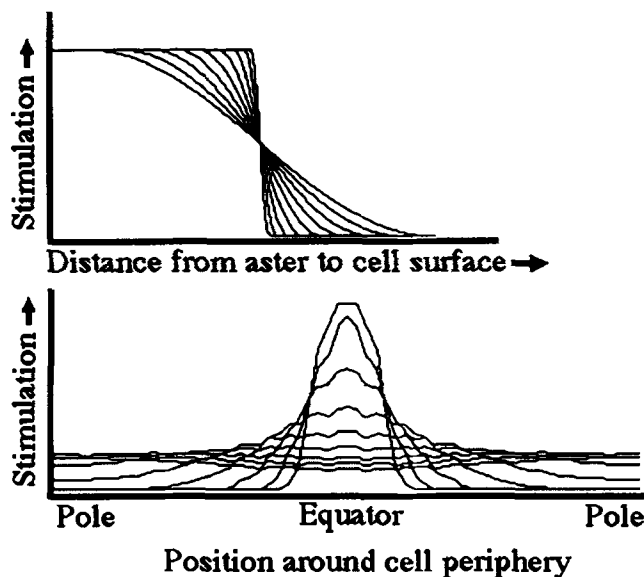


Figure 9. Relative amounts of net aster signal at the surface of a spherical cell, depending on how sharply the sigmoidal signal function falls off with distance. The sharpest drop-off results in the highest peak of equatorial stimulation.

lying in the approximate envelope shown in Fig. 5. Considering the case of asters near the relatively flat surface of an egg cell (e.g., *Drosophila*), if the MA is parallel to the cortex and the asters are a distance, L , apart, at a depth, d , away from the cortex, then the function would need to drop off at a distance greater than the square root of $d^2 + L^2/4$ but less than the square root of $d^2 + L^2$.

The simulations also show that successful furrow induction should also depend on the sharpness with which the drop-off occurs in these curves. This dependence is illustrated in Fig. 9. The more gradual the decrease in effect beyond the threshold distance, the lower and less sharp will be the resulting peak of cortical contractility.

Consequences of Changing the Distance between the Asters

The simulation programs were also used to predict the consequences for furrow induction of changing the distance between the aster centers. The consequences were compared both for the case of inhibitory aster signals varying as the inverse square of distance and for the case of stimulatory aster signals assigned to vary according to the sigmoidal function.

The predicted consequences for moving the asters closer together were rather similar for both functions: a broadening and flattening is produced both in the equatorial maximum resulting from the stimulatory sigmoidal function and in the equatorial minimum which results from the inverse power function. Thus, in both cases, the computations predict that furrow induction should be inhibited by moving the asters closer together. Rappaport and Rappaport (1984) have described the inhibition of furrow induction by treatment of cells with urethane, which has the effect of shortening the microtubules that radiate from the asters as well as the distance between the asters.

In contrast, when the effects of moving the asters farther apart are calculated, the predicted results differ considerably

depending on which function is used. For the inverse power function, the result is a deepening of the equatorial minimum, corresponding to the prediction that furrow induction should be favored and certainly not inhibited. On the other hand, when the aster effects are made to vary with distance according to the sigmoidal function, the result of increasing the distance from one aster to the other is a progressive flattening and narrowing of the equatorial maximum, which eventually disappears when the distance between the asters has been increased sufficiently. However, these equatorial maxima, which have been destroyed by moving the asters too far apart, can be "rescued" by constricting the equator of the cell inward. Rescue can also be accomplished by a proportional increase in the distance beyond which the aster effects begin to decrease, however.

Other Distortions of Cell Geometry and Their Expected Consequences

Many other shape distortions were examined using the computer programs. In particular, we looked for those cell shapes in which the sigmoidal curve would be least able to produce equatorial maxima of contractility and, thus, to induce cleavage furrows. We found that the surface contour to which that mechanism ought to be most vulnerable would be one in which the equatorial part of the cell is made to bulge outward away from the MA. If the equator is bulged outward to a sufficient degree, then the production of the equatorial maximum will be prevented and furrow induction ought, therefore, to be blocked. How much bulging is sufficient to produce this effect depends on the sharpness with which the sigmoidal curve drops off: the faster the decline in effect past the threshold, the less the equator will need to be bulged out to destroy the equatorial maximum.

Eventually maxima come to lie at the "corners" where the cell surface bends outward at either side of the bulge. Thus, the calculations predict that, in such cells, furrow induction should either be prevented entirely or that the furrows should form at one or both of these corner areas just to either side of the equatorial bulge. Rappaport has observed that cells distorted in these ways do cleave, but that the furrows form just to one or the other side of the bulge (though never with two furrows, one on either side, so the predictions of the model are only partly confirmed).

Effects of Interposing Obstacles to the Aster Signals

Rappaport and Rappaport (1983) have tested the effect of inserting physical barriers between the MA and the cell cortex before cleavage. Their result has been that obstacles placed between the MA and the cell poles fail to disturb the induction of equatorial furrows, but objects inserted between the MA and the equatorial regions do block furrow induction. It hardly requires computer simulations to show that these results are consistent with the hypothesis that furrows form where the cortex is most stimulated and are the opposite to what should occur if furrows formed where cortex contractility is least inhibited. Nevertheless, a few such simulations were conducted. The results are consistent with stimulation but are contrary to what would be expected if the signals were inhibitory.

We have also examined the question of whether the asters themselves might play the role of obstacles, blocking the sig-

nals from the other aster. Logically, it seems plausible that asters would have such a blocking effect; yet all the calculations and mapping of the aster effects up to this point (this paper and White and Borisy, 1983) have been made as if the asters were transparent to one another's signals. We therefore conducted additional calculations in which effects passed within a chosen distance of the opposite aster center were excluded. The results of astral blocking are consistent with the induction of equatorial furrows, both in the case of the inverse power inhibitory signals and in the case of the sigmoidally decreasing stimulatory signals. In the case of the sigmoidal stimulatory function, this masking produces no effect because the signals that would have had to pass through the other aster would never have reached the cortex anyway (the effects have dropped to zero at that range). On the other hand, when the aster effect is made to vary with the inverse square or other power of distance, then shadowing effects by the asters have the effect of adding a pair of small inflections to the curve mapping the relative amounts of inhibition around the cell periphery. An extreme interpretation might be that one should therefore have predicted the formation of a pair of supernumerary cleavage furrows at these points; but that would only be expected if these shadows from the asters have extremely sharp edges (with total blockage out to a certain distance and no blockage beyond that). A more blurry effect seems more realistic and would not contradict the observed behavior of cells.

Discussion

We find that the predictions of both theories of cleavage furrow induction depend crucially upon the mathematical rules by which the aster's effects decrease with distance. Although we confirm White and Borisy's (1983) finding that inhibitory signals could induce equatorial furrows in spherical and elliptical cells, we find that signals obeying these rules would fail to induce normal equatorial furrowing if the cell is distorted into a cylindrical shape, its surface is flattened, or its equator is artificially constricted. In view of Rappaport's experimental demonstrations that distortion of cell shapes in these ways does not block cleavage (Rappaport, 1960, 1964, 1981, 1985, 1988), we conclude that furrow induction cannot be the result of such an inhibition mechanism.

On the other hand, our results support the idea that stimulatory signals (whose effect is to strengthen cortical contractility rather than to inhibit it) would be able to produce maxima of cortical contractility at the observed sites of furrow induction; we find that such signals could accomplish this not just in spherical cells but also in cylindrical, flattened, and constricted cells. We even find that stimulatory signals can induce the hexagonal pattern of acto-myosin accumulation that is observed in the mass cleavage of the *Drosophila* embryo. Our simulations showed, however, that these various results would occur only when the stimulatory effects vary with distance according to functions of a rather narrow range of sigmoidal shapes in which the effect is relatively constant out to a threshold distance but then decreases sharply. And, when this threshold distance is made either too large or too small (relative to other cell dimensions), then furrow induction will fail.

Our results thus support Rappaport's theory of furrow induction by a stimulatory signal from the asters but they also

raise questions about the means by which the range of this signal can be adjusted according to the dimensions of a given cell, as would be necessary for it to accomplish its function. One possibility would be for this threshold distance to change gradually during the time before cleavage until it reached the correct dimensional "window," inducing the aggregation and alignment of the contractile proteins at the correct location. In principle, either a gradual increase or decrease in range could be effective; but only the former possibility would correctly predict what is observed when the MA is displaced closer to one equatorial surface than the other (as is normal in coelenterate eggs; Rappaport and Conrad, 1963) because these cells form furrows first on the side closest to the MA.

As to the physical or chemical nature of the signals passing from the asters to the cell cortex, simulation studies can provide only the most indirect inferences. This is because any mechanisms that cause effects to vary with distance according to curves of the same shape will necessarily make exactly the same predictions about where and whether cleavage furrows ought to form; and the similarity of prediction will hold regardless of the geometric distortions imposed on the cell surface or on the positions of the asters. Furthermore, it turns out that equally indistinguishable consequences would follow even from two different types of signals, one stimulatory and the other inhibitory, in the special case where the curve describing one happened to be an exact mirror image (in the vertical direction) of the curve describing the other. To take the most relevant example, an inhibitory signal that increased sharply in effect beyond a certain threshold distance and then remained constant at greater distances would produce the same spatial patterns of relative cortical contractility as those produced by a stimulatory signal obeying the sigmoidal function discussed above. Nevertheless, in view of the recent direct evidence that single asters stimulate increases in cell contraction (Rappaport and Rappaport, 1985, 1988), it seems safe to dismiss the possibility of an inhibitory signal that increases with distance.

The shape of the curve by which these signals vary with distance should depend on the physical or chemical mechanism that is conveying this signal, and the shape should therefore be an important clue as to the nature of the signal. Unfortunately, both of what would otherwise seem to be the most plausible signaling mechanisms, namely chemical diffusion and the radiation of the microtubules from the aster centers, happen to predict rather similar patterns of variation with distance (approximating the inverse square curves). This would be true not only for chemical diffusion gradients (Bossert and Wilson, 1963) but also for the local densities of linear elements (such as microtubules) if these radiate outward from the asters at approximately equal angles. Thus, in view of Asnes and Schroeder's (1979) observations of the distributions of microtubules in cleaving sea urchin eggs, one would expect that aster effects mediated by microtubules alone would be maximal near the poles rather than the equator. However, as Devore et al. (1989) show in the accompanying paper, it is possible to explain many aspects of furrow induction using a hybrid mechanism that combines radiating microtubules with diffusion of a substance from the ends of these tubules.

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