INVITED REVIEW

A minimal mechanism for organizing the events of cytokinesis

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The importance of cytokinesis in the life of the cell was recognized long before the role of chromosomes was understood. The association between mitosis and cytokinesis was obvious. Some investigators considered the two processes coincidental and not causally related while to others it seemed logical to assume that the mitotic apparatus is physically involved in both processes. Animal cell division is not difficult to observe, especially during the early cleavage divisions of marine invertebrate embryos, and by a century ago scores of hypothetical mechanisms had been proposed. Among these was a group of theories that postulated that the linear non-chromosomal elements of the mitotic apparatus (which we now recognize as microtubule bundles) by one means or another moved and arranged the chromosomes and subsequently deformed and divided the cell periphery. These hypotheses were attractive because they were parsimonious, they seemed to be consistent with the observable process, they were easy to understand and they unified two complex events. They became untenable, however, after it was shown that cytokinesis could occur after the mitotic apparatus had been dissolved [2] or mechanically removed [4] while the cell was still spherical.

It was well known that the position and orientation of the plane of division was correlated with the position and orientation of the mitotic apparatus even after it had been moved, and the idea that the mitotic apparatus regionally changed the behavior and physical properties of the cell periphery so that it could deform and divide the cell was generally accepted. It was now apparent that not only was the physical nature of the division mechanism unknown, but there was also a new set of mysteries concerning the ways in which the division mechanism is oriented, activated, organized and terminated. No observational method at any level of complexity proved capable of providing decisive information about these events, but, fortunately, experimentation on dividing echinoderm eggs (which had been the subject of investigation for many years) produced some insights. As investigations progressed it became apparent that the positioning, timing, rate of progress and termination of the division mechanism were affected by the geometrical relation between the mitotic apparatus and the surface. Experiments in which these geometrical relations were changed without taking anything from or adding anything to the cell have yielded information concerning both the physical nature of the process and the events that set it in motion.

The events of cytokinesis that have been investigated are linearly arranged and very regularly spaced in both mitotic time and real time. Since the entire process in the dividing sand dollar egg is completed in eleven minutes, some of the sub events are very brief. Abnormal timing and positioning of division activity are relatively rare and the careful observer is left with the thought that either there are precisely functioning controls or that the system is flexible and self-correcting. Since division can occur in the absence of a spindle and chromosomes [6] the possibility that its events are orchestrated by mitotic events appears remote.

In sand dollar eggs the part of the cell periphery that becomes the specialized contractile ring acquires its unique quality by reason of its geometrical relation to the mid region of the mitotic apparatus. The process that alters the future contractile region requires one minute [16]. During the ensuing four minutes there are no visible changes while some reorganization is assumed to take place. At the completion of the latent period, the affected equatorial region flattens and then indents as a consequence of circumferential contraction. Ultrastructural changes then develop at the base of the furrow [20] [21] and the cross sectional area of the altered region decreases slightly as the circumference decreases [21]. This relationship results in a steady decrease in the volume of the

ultrastructurally modified contractile ring. Eight minutes after division begins, it is completed with the formation of two identical nucleated cells lacking any vestige of the contractile ring. The superficial similarity between the division of echinoderm eggs and the behavior of manipulated oil drops and soap bubbles has led to the suggestion that surface tension forces could create furrows in response of folding and distortion. Experimental tests of this possibility yielded uniformly negative results [14].

The location, the timing and the duration of the events of cytokinesis in sand dollar eggs can be modified by relatively simple micromanipulation [19]. The position and orientation of the division plane in the sand dollar egg can be located any place in the cell if the mitotic apparatus is repositioned early enough in the cell cycle. Although the spindle and chromosomes are not essential [6], the geometrical relation between the asters, located at the ends of the spindle, and the equatorial cell surface are critical. Aster pairs become incapable of establishing furrows if they are too widely separated or too distant from the equatorial surface. Aster pairs that fail to establish furrows when they are widely separated can establish furrows when they are pushed closer to the surface [8]. When the distance between asters is increased 8.6 μm or more greater than normal, the rate of furrow progress is slowed [13]. The rate of furrow progress is also decreased when the distance between the mitotic apparatus and the surface is increased [11]. When the mitotic apparatus is moved closer than normal to the equatorial surface cytokinesis begins earlier than normal [19]. These and other similar experimental results suggest that important properties of the division mechanism are determined by the geometrical relation between the mitotic apparatus and the surface that exists when they interact.

It now appears that the role of the mitotic apparatus in cytokinesis is to transport an agent from the central regions of the cell to the periphery. The unequal distribution of the agent or stimulus can then create a pattern of unequal tension at the cell surface which results in deformation [15]. The rate of stimulus movement can be estimated from the time delay of furrow appearance that occurs when the distance between the surface and the mitotic apparatus is manipulated. The estimated rate is 6.3 μm/min [9] which roughly approximates the rate of microtubule elongation [1]. The time of furrow formation has been advanced as much as 7.1 min by positioning the mitotic apparatus and the surface abnormally close [19]. Because all the events of normal cytokinesis can be completed in 13 min, the relative magnitude of the advance is appreciable. This experiment also demonstrates that the stimulus is present in the mitotic apparatus and functional, and that the surface is capable of response significantly earlier than their interaction normally occurs. It is implied that neither the time when the stimulus develops nor the time when the surface becomes responsive determines when division begins. Rather, the event is triggered when the mitotic apparatus and the surface are brought together by the extension of mitotic apparatus microtubules which normally elongate at that time [3].

The first indication of a change in the behavior of the surface is the development of a concentrated band of granules that appears in the equatorial region of pigmented sea urchin eggs. The pigment granules are firmly embedded in the cortex at that time, and it is believed that the band's appearance is the consequence of regional cortical contraction [24] [17]. After the equatorial region flattens and then indents, an array of circumferentially oriented actin microfilaments as well as associated myosin appears immediately subadjacent to the equatorial surface membrane [23]. The apparent volume decrease of the contractile ring has been hypothetically attributed to a return of actin to a cytoplasmic pool after its interaction with myosin [22]. As the cell is constricted during division the dimensions of the mitotic apparatus change as does the geometrical relation between the mitotic apparatus and the deformed cell surface. The distance between the asters increases and at the same time each single aster becomes increasingly surrounded by an increasingly spherical surface. A spherical cell containing two asters and no spindle and chromosomes will divide, but a spherical cell containing a single aster will not [5]. However when a cell with a single aster is reshaped into a cylinder, a furrow forms in the plane of the aster [18]. It would appear from this and other evidence that the early stages of cytokinesis disrupt the geometrical relations that

normally exist when the mitotic apparatus and the surface interact. The duration of the period when the stimulus is effective and the surface is responsive was determined by reshaping cells into cylinders so that the mitotic apparatus is oriented parallel to the cylinder's long axis. Each time a furrow formed in the normal relation to the mitotic apparatus, the mitotic apparatus was moved to another position. In this circumstance multiple furrows form after each repositioning of the mitotic apparatus over a period of 18 min [12]. This means that the mitotic apparatus is effective and the surface is responsive about twice as long as the cell normally requires for duration of division activity (Fig. 1).

In normal sand dollar cleavage, the functional life of the division mechanism, the period from the first indication of furrow constriction to the separation of the two daughter cells is 8 min long. It is possible to increase the dimension of the region to be divided by reshaping the egg into an elongate ellipse and moving the mitotic apparatus to one end of the ellipse so that the furrow must divide the ellipse parallel to its longest axis. In this circumstance the furrow indents from one end of the cell and progresses toward the other. Furrowing is never completed but the furrow continues to deepen for 21.8 min. The contractile mechanism in sand dollar egg cleavages normally functions for 8 min or 17% of the 48 min cycle. Taking into account both early (4.9 min) and prolonged (21.8 min) experimentally induced cleavage, the total period when cleavage was possible was 26.7 min or 56% of the cell cycle [19].

The tension exerted by a cleavage furrow in isometric contraction can be estimated by measuring the bending moment of a calibrated glass needle inserted through and perpendicular to the division plane [7]. In sand dollar eggs the maximum force exerted by both the first and second furrows is 1.5 x l0-3 dyne. At second cleavage one of the two cells produced by the first cleavage can be used as a time control. When the completion of cleavage was mechanically blocked in the experimental cell, its furrow continued to exert maximal tension up to 9 min after the time control had completed division [10].

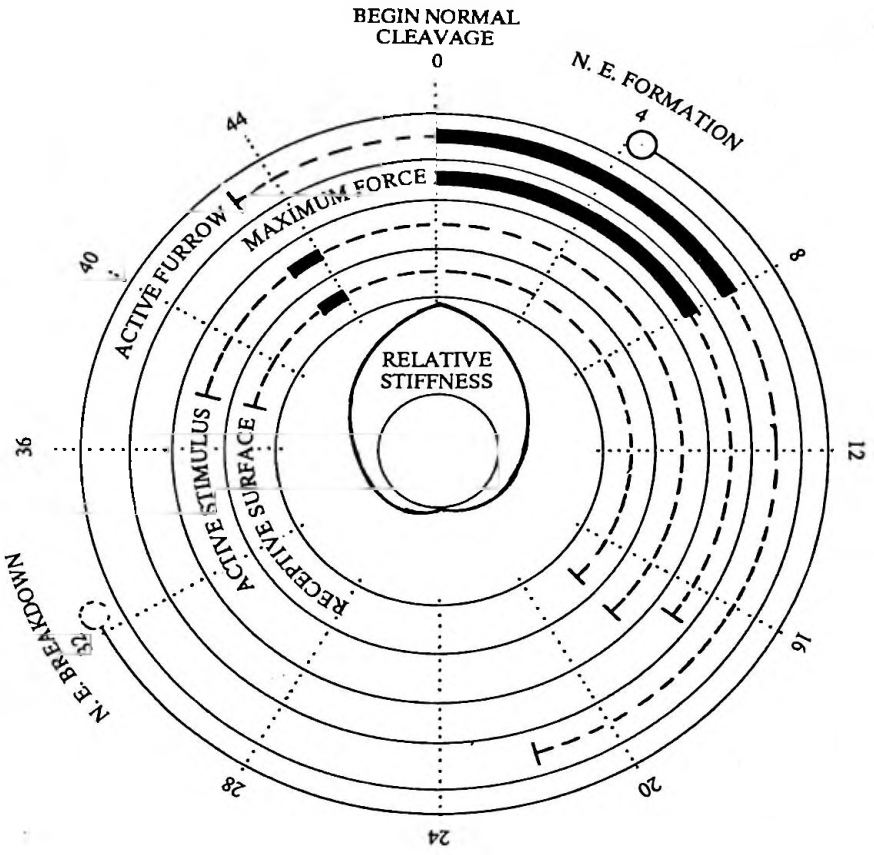

Figure 1. Summary of time relations among division-coordinated events in the second and third cleavages of sand dollar eggs. The complete cycle requires 48 minutes. Data for maximum force were taken from [10]. The data for relative stiffness were taken from [25]. The data for active furrow, active stimulus and receptive surface were taken from [19].

Dark bar = normal duration or minimum time necessary for activity. Dashed line = time during which event can occur. NE = nuclear envelope. From [19] and with the permission of Academic Press.

It appears that sand dollar cleavage cells are prepared to carry out simultaneously a number of the actions and interactions that lead to and accomplish cytokinesis over a period of roughly half of the duration of the cell cycle (Fig.1). But in reality these events are sequential, their durations are predictable and variable, and they occur during about 25% of the cell cycle. Lest we forget Occam's razor, I must point out that it is possible to propose a simple hypothetical explanation of the orderliness of these events in which subtle molecular switching processes are not required. Briefly stated, activities may lose their effectiveness or terminate by reason of the consequences of their functioning. For example, the cleavage furrow forms where it does when it does because the elongating microtubules of the mitotic apparatus can elicit regional differences in contractility of the cell surface. The regionality of the contractility is the consequence of the geometrical relation between parts of the mitotic apparatus and the surface. After one minute, the pattern is established. In 4 minutes the form of the cell is changing and the distance between the asters continues to increase. In consequence, the geometrical relation between the mitotic apparatus and the surface is no longer that which normally elicits regional contraction even though the surface is still responsive and the parts of the mitotic apparatus are still effective. At that time, their continued competence can only be demonstrated by experimental manipulation. In similar fashion, the disappearance of the contractile ring at the end of division can be attributed to its function if one accepts the idea that the

linear elements of the ultrastructure revert to a disorganized cytoplasmic pool after they participate in force production [22]. The persistence of the furrow when its progress is blocked with needles or the amount of cytoplasm to be divided is increased is consistent with the idea that the ability of the cell surface to participate in division activity does not suddenly cease when division is normally completed. It appears that before division begins the cell is in a state of general readiness. The triggering of events is associated with the development and distribution of the microtubule elements of the mitotic apparatus. Thereafter the sequence and duration of events are determined by the nature of the events themselves. Geometric relations are important, and it may be relevant to remark that the apparent regularity of cleavage cell divisions is correlated with their relative uniformity of size and shape, and their physical isolation from other cells. Although the simplest and most logical hypothetical mechanisms of cell division rarely survive experimental test, it is tempting to think that if cells do not rely on such a mechanism to orchestrate the events of cytokinesis, they should try it.

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