Ray Rappaport Chronology: Twenty-Five Years of Seminal Papers on Cytokinesis in the Journal of Experimental Zoology

THOMAS D. POLLARD*
*Departments of Molecular Cellular and Developmental Biology and Cell Biology
Yale University, New Haven, Connecticut*

Between 1960 and 1993 Ray Rappaport (Fig. 1) published a series of seminal articles in the Journal of Experimental Zoology that defined the fundamental rules for how the mitotic apparatus determines the position of the cleavage furrow. This lasting achievement is recognized in every cell biology textbook and his story is told in every cell biology class. His legendary experiments remain fresh and his papers still make exciting reading. This appeal comes in part from Ray's gift as a storyteller, but it's mainly the events in these riveting stories that hold one's attention. The work is so important that his strategies and ideas strongly influence current research on cell division. We still debate the meaning of his experiments.

This remarkable accomplishment was achieved in a manner that has been all but lost in contemporary biological research. Ray did virtually all of his experiments with his own two hands during summers at the Mt. Desert Island Biological Laboratory in Salisbury Cove Maine. During the rest of the year he taught at Union College in Schenectady New York. The Mt. Desert Island Biological Laboratory is a rustic collection of wooden buildings, a world apart from the pretentious palaces designed by signature architects that house many biological research labs today.

Ray's equipment was as modest as the lab in Maine. It consisted of a Unitron inverted microscope, a deFonbrune microforge, a dissecting microscope, micromanipulators, a simple centrifuge, and various observation chambers that he fabricated himself from materials available at a hardware store. Ray used a rowboat to collect sand dollars from the local waters for his experiments. He never used an electron microscope or a fluorescence microscope. The work was done without large research grants, graduate students, postdoctoral fellows, or research assistants. A couple of undergraduates helped with two early papers. Ray's wife Barbara was a constant source of support, helped with writing, and co-authored some later papers.

Although Ray never worked with a gene, protein, or antibody, he defined the rules for how the mitotic apparatus stimulates the cortex to form a furrow. He showed that the asters of the mitotic spindle are the source of this positive signal (at least in echinoderm eggs), measured the rate that the stimulus moves from the mitotic asters to the cortex, identified the time required for the stimulus to make a lasting impression on the cortex, and discovered a latent period between receipt of the signal and the formation of a visible

Fig. 1. Photograph of Ray Rappaport in 1989.

*Correspondence to: Thomas D. Pollard, Departments of Molecular Cellular and Developmental Biology and Cell Biology, Yale University, New Haven, CT 06520-8103. E-mail: thomas.pollard@yale.edu

furrow. His work is full of implications regarding molecular mechanisms, but he did not concern himself with molecules. In fact microtubules are essentially the only cellular structure mentioned in his papers. Only in his 1965 paper did he mention molecules: "During the apparently quiescent 2.5 minute period after stimulation, changes at the molecular level that culminate in visible furrowing must occur."

I will review Ray's accomplishments with short summaries of my favorite Rappaport papers that appeared in the *Journal of Experimental Zoology* (plus one from *Science*). I trust that this chronology will reveal the elegant simplicity of Ray's experimental design and the compelling logic of his interpretation. Readers of the original papers will discover Ray's scholarship, since he always modestly acknowledged his debt to the experimental biologists from the late 19th century up to 1960, whose work inspired his own.

Experiments concerning the cleavage stimulus in sand dollar eggs. 1961. J. Exp. Zool. 148:81–89. R. Rappaport.

Ray began by recalling that although the mitotic apparatus was considered to drive the formation of the cleavage furrow, little was known about the part of the mitotic apparatus producing the signal for furrowing or the part of the cortex receiving and transducing this signal. He aimed to find the source of the signal by removing parts of the mitotic apparatus from a cell and observing the consequences for cytokinesis. He selected sand dollar eggs for his experiment, since they are optically clear and thus favorable for observing the mitotic apparatus and the cleavage furrow. At 20°C these cells initiate cytokinesis about 90 minutes after fertilization. In a classic experiment he indented the surface of sand dollar eggs 20 minutes after fertilization with a glass sphere on the end of a needle. The sphere was pressed through the cell into contact with the supporting slide, creating a torus (donut) shaped cell. The first cleavage occurred at the normal time producing a "horseshoe shaped" cell with two nuclei (Fig. 2). During the second mitosis the two mitotic apparatuses drove the formation of furrows that "cut cells from each of the two free ends of the horseshoe. The two free cells thus established were uninucleate but the binucleate cell, representing the bend of the horseshoe, contained an aster and a nucleus from each of the mitotic figures. The asters in the binucleate cell moved
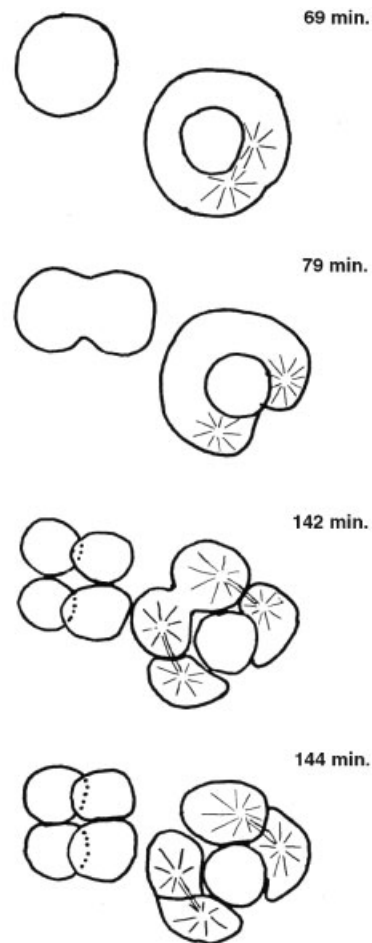

Fig. 2. Reproduction of the drawing showing the famous experiment on cytokinesis in a torus-shaped sand dollar egg (right) compared with a control egg (left). From R. Rappaport, J Exp Zool 148:81–89, 1961.

closer together as the two cleavages were completed. Another furrow appeared between the asters in the binucleate cell and was completed in normal time. The embryo was thus converted to a 4 cell stage." This must have been a magic moment! Ray called this "furrow between asters that were not joined by a spindle" a "non-spindle furrow." He concluded, "following numerous repetitions of this experiment it became clear that the furrow could appear between any two astral regions regardless of the orientation of the mitotic figures involved." This remains the best evidence that the asters of the mitotic spindle are a source of signals that stimulate the cortex to form a cleavage furrow. Forty years later, we still do not know the molecular nature of this signal, but Ray's insights continue to motivate current workers.

Ray also confirmed an observation made by E.B. Harvey ('35) on sea urchin eggs. If one centrifuges fertilized eggs just as the first furrow starts to form, the force can displace the mitotic apparatus in some cells to a new location, where it can stimulate the formation of a second cleavage furrow. Ray noted that Kawamura ('60) got a similar result moving the mitotic apparatus of grasshopper neuroblasts and he concluded "it may be that all parts of the astral surface are capable of delivering the stimulus...but only in the zone of confluence of the asters would stimulus activity be sufficient to elicit a furrow.'' He noted that a ''constriction hypothesis (Lewis, '42; Marsland and Landau, '54) is the simplest such mechanism that has been proposed. No evidence either inconsistent with or directly insubstantiating a constriction mechanism has yet been produced.''

This elegant paper set the course for Ray's research for the next 30 years, which established the importance of pairs of asters stimulating contraction in the furrow by sending a positive signal to the adjacent cortex.

An experimental analysis of unilateral cleavage in invertebrate eggs. 1963. J. Exp. Zool. 153;99–112. R. Rappaport and G.W. Conrad

In this work Ray and Gary Conrad, an undergraduate student on a summer fellowship, set out to distinguish between the two leading hypotheses for how the mitotic apparatus stimulates cleavage furrows. Do the asters stimulate active constriction of the furrow through a positive signal or does the absence of a positive signal relax the cortex at the poles of the cell? Their strategy was to examine the ''geometrical relations'' of the mitotic apparatus to the cortex in cells with different types of furrows. They compared cytokinesis in a coelenterate egg, which has an eccentrically positioned mitotic apparatus and normally divides ''unilaterally'' beginning at the point where the surface is closest to the midzone of the spindle, and cytokinesis in an echinoderm (sand dollar) egg, where the mitotic apparatus is usually located centrally and the furrow forms simultaneously around the entire equator. They used microsurgery to create a coelenterate egg with a central mitotic apparatus, which then divided like a sand dollar egg with a circumferential furrow. They used compression to create a sand dollar egg with an eccentric mitotic apparatus, which divided unilaterally like a coelenterate egg.

Since these two strategies for furrowing depended only on the position of the mitotic apparatus, they concluded that geometry rather than species-specific features of the mitotic apparatus or the cortex determines the pattern of cleavage. They also argued that none of these experiments is consistent with polar relaxation due to the absence of a signal from the asters. Instead they favored the older hypothesis that the furrow forms by contraction of the cortical cytoplasm.

A simple manipulation of compressed sand dollar eggs dividing with unilateral furrows laid the ground work for later experiments. They found that inactive cortex far from a mitotic apparatus can initiate a furrow in a ''few minutes if pushed close to the mitotic apparatus.'' This confirmed that proximity of the mitotic apparatus to the cortex is important for communication of the furrowing signal and that brief exposure to this signal is sufficient to initiate a furrow. They also confirmed microsurgery experiments described by Yatsu ('12) demonstrating that furrows are self-propagating in coelenterates even in the absence of a mitotic apparatus. They extended this observation to echinoderms.

Geometrical relations of the cleavage stimulus in constricted sand dollar eggs. J. Exp. Zool. 155:225-230. 1964. R. Rappaport

Previous work showed that ''although the source of the stimulus lies in the asters, no furrow appears if the distance between the asters is too great and when ...the mitotic apparatus is....eccentric, the furrow always appears first in the surface closest to the zone between the asters.'' Here Ray set out to ''determine how the asters create different intracellular environments at the poles and the equator of the cell.'' In particular he aimed to learn if the asters work by sending a ''stimulus which affects the poles but fails to reach the presumptive furrow.'' The experiment was to remove part of the extracellular fertilization envelope from a sand dollar egg, creating a dumbbell shaped cell with half of the cell protruding through the hole and the other half within the fertilization envelope. In cells with the mitotic apparatus centered across the constriction, all parts of the cortex were equidistant from an aster. Such cells still divide normally, ruling out mechanisms where furrowing depends on the absence of a signal from the asters, as proposed by Mitchison and Swann ('58) and Wolpert ('60).

Duration of stimulus and latent periods preceding furrow formation in sand dollar eggs. J. Exp. Zool. 158:373–382. 1965. R. Rappaport and R.P. Ebstein

Ray and an undergraduate summer student R.P. Ebstein set out to determine the rate that signals move from asters to the cortex to stimulate a cleavage furrow. They used three methods. The first method (after Harvey, '35) involved centrifuging fertilized sand dollar eggs for three minutes just at the time of furrowing. Subsequently two furrows appeared in some cells. The location of the mitotic apparatus prior to centrifugation determined the position of the first furrow. After about 10 minutes from the onset of centrifugation, a second furrow formed between the asters of the repositioned mitotic apparatus. These events usually produced three cells, only two with nuclei, unless the primary furrow regressed.

In the second experiment they used needles to move the mitotic apparatus in the cytoplasm of cells about to divide. After about 2 minutes in a new location, the mitotic apparatus stimulated a new furrow located between the asters. This maneuver could be repeated, producing up to three furrows in one cell.

The third experiment was to displace the mitotic apparatus to one side by flattening cells with a needle. The eccentric mitotic apparatus stimulated the adjacent cortex to form a "unilateral furrow" lying in a plane between the asters. Once this furrow was under way, the opposite side of the cell could be pushed with a needle toward the mitotic apparatus for various times. Exposure of the cortex to the mitotic apparatus for 1 minute or more resulted in the formation of a furrow, even if the needle were withdrawn and the test cortex returned to its original position far from the mitotic apparatus. Following 1 minute of stimulation, furrowing began after a delay ("latent period") of 2.5 minutes.

They concluded that "during the apparently quiescent 2.5 minute period after stimulation, changes at the molecular level that culminate in visible furrowing must occur." They noted that the equatorial cortex becomes more resistant to deformation during the 2 minute latent period and then overtly constricts. They wondered if these are manifestations of the same process. Since the cytoplasm under the cortex can be moved after receipt of the stimulus from the mitotic apparatus, the constriction of the cortex must be "independent of any subsurface cytoplasmic configuration."

Cell division: direct measurement of maximum tension exerted by the furrow of echinoderm eggs. Science 156:1241–1243. 1967. R. Rappaport

Ray inserted a pair of needles into dividing sea urchin eggs to measure the force produced by the furrow. A thick needle resisted deformation. A thin calibrated needle, was used to measure force. The furrow constricted between these needles until they blocked further contraction. The thin needle bent, revealing a maximum force on the needles of about 0.002 dynes independent of the circumference of the furrow. After Schroeder ('72) measured the cross sectional area of the contractile ring in echinoderms (0.2×8 μm), it was possible to calculate that the tension produced is about 2.5×10^5 dyne/cm^2, a small fraction of the force per cross sectional area produced by striated muscles. This experiment established that the furrow (later interpreted to be the contractile ring of actin filaments and myosin-II) generates force sufficient to deform the cell surface and to account for constriction during cytokinesis.

On the rate of movement of the cleavage stimulus in sand dollar eggs. J. Exp. Zool. 183:115-120. 1973. R. Rappaport

In this paper Ray uses an exceedingly simple experimental design and a results section consisting of just 108 words to measure the rate that the furrowing stimulus moves from the mitotic apparatus to the cortex. He flattened fertilized sand dollar eggs with two "stout" glass needles and allowed them to proceed through mitosis and cytokinesis. In such flattened cells furrows form on the lateral edges, rather than on the compressed surfaces. In cells with an eccentric mitotic apparatus, the furrow formed first on the side closer to the mitotic apparatus. The more distant cortex responded later. The delay between these two events was proportional to the difference in the distance between the mitotic apparatus and the two sides of the cell (Fig. 3). Ray interpreted the slope of 6.3 ± 1.8 μm per minute to be the rate that the stimulus moves from the mitotic apparatus to the cortex. He noted that asters are radial arrays of microtubules and that particles had been observed to move centrifugally along microtubules at this rate. He concluded that "studies of centrifugal movement in asters would be desirable." Since we still do not know the molecular nature of the stimulus, such experiments have yet to be done.
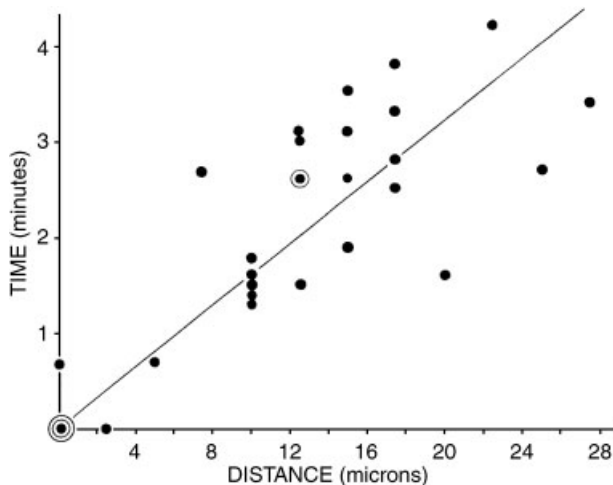
Fig. 3. Reproduction of Figure 2 from R. Rappaport, J Exp Zool 183:115–120, 1973, showing "The relation between the difference in distance between the spindle and the near and distant cell margin, and the difference in time between the appearance of the furrow in the near and in the distant margins."

Cytokinesis: cleavage furrow establishment in cylindrical sand dollar eggs. J. Exp. Zool. 217:365–375. 1981. R. Rappaport

Studies by Hiramoto and others dating from 1940–1975 had shown that cleavage proceeds if the mitotic apparatus is removed or destroyed after anaphase onset. Thus, in the time before anaphase an irreversible interaction occurs between the mitotic apparatus and cortex. Ray used freehand manipulation under a dissecting microscope to insert sand dollar eggs into short segments of a glass capillary. The cells divided earlier than spherical controls, presumably owing to the reduced distance between the mitotic apparatus and the cortex. If the mitotic apparatus was removed 4 minutes or less before a furrow formed in a matched control, a furrow still formed in the operated cell. Therefore the effect of the mitotic apparatus on the cortex becomes irreversible about 4 minutes before the first sign of a furrow. No furrow forms if the mitotic apparatus or even a single aster (first shown by Hiramoto, '71) is removed more than 5 minutes before the furrow would form. Vigorous disruption or removal of the cytoplasm between the poles of the spindle and the polar cortex had no effect on furrowing. Nor did disrupting the mitotic apparatus by spiking from end to end with a needle. Nor did sucking both poles of the mitotic apparatus into pipettes without breaking the plasma membrane. Ray

concluded that the geometry of the mitotic apparatus (especially the asters) relative to the cortex is key to signaling for cytokinesis. I find it interesting that Ray's discussion tended toward a physical rather than chemical interpretation for cause and effect in this intracellular signaling system, in spite of negative results in his own experiments with extreme physical manipulation of the spindle and the polar cytoplasm.

Cytokinesis: The effect of initial distance between mitotic apparatus and surface on the rale of subsequent cleavage furrow progress. J. Exp. Zool. 221:399–403. 1982. R. Rappaport

The 1973 paper examined the effect of the distance between the mitotic apparatus and the cortex on the time required for a stimulus to initiate a cleavage furrow. In this paper Ray showed that the distance between the mitotic apparatus and the surface also influences the rate that furrows progress once they get started. He used flattened, fertilized sand dollar eggs (diameter 175 μm, thickness 60 μm) with eccentric mitotic apparatuses. The rate of furrowing was inversely related to the distance between the mitotic apparatus and the cortex. Ray also used the difference in the onset of furrowing on the two sides of eccentric mitotic apparatuses to re-measure the rate that the stimulus moves from the mitotic apparatus to the cell surface. The rate of 7.4 ± 3 μm/minute is similar to his 1973 paper. He concluded that the effect of the mitotic apparatus on the cortex falls with distance.

Repeated furrow formation from a single mitotic apparatus in cylindrical sand dollar eggs. J. Exp. Zool. 234:167–171. 1985. R. Rappaport

Ray trapped fertilized sand dollar eggs in a silicone tube, where the cylindrical cells form furrows. He moved the mitotic apparatus with probes inserted into the ends of the tube. If the mitotic apparatus was moved to a new position just after the first furrow appeared, that furrow began to regress after about 30 seconds and a new furrow appeared between the repositioned asters after 1 to 2 minutes. One mitotic apparatus could stimulate the formation of up to 13 transient furrows over about 25 minutes. Only 2 minutes was required to stimulate a new furrow with this geometry, presumably because the asters were much closer to the cortex than in a round cell.

If the mitotic apparatus was moved only short distance, the constriction would "slide" along the cortex to the site of the new secondary furrow. These experiments showed that the mitotic apparatus remains active for furrow stimulation long after cytokinesis normally begins.

Surface contractile activity associated with isolated asters in cylindrical sand dollar eggs. J. Exp. Zool. 235:217–226. 1985. R. Rappaport and B.N. Rappaport

Ray confined fertilized sand dollar eggs in silicone rubber tubes. Sucking a cell into and out of the tube multiple times sometimes fractured an aster from the mitotic spindle. This aster could be removed entirely or pushed about in the cytoplasm. If pushed close to the rest of the mitotic apparatus, a normal aster developed. A single aster in a cylindrical cell could stimulate contraction of the nearby cortex at the same time that a furrow would have developed in a cell with two asters. The response to single asters ranged in intensity from minimal and transient local contractions to a deep furrow. Ray pointed out that cortical contractions stimulated by a single aster are inconsistent with polar relaxation theories. (Note that Ray was still battling against polar relaxation theories, 24 years after his opening salvo!)

CONCLUDING OBSERVATIONS

If you enjoyed this brief account of a life in science and would like to learn more about cytokinesis, try Ray's authoritative book "Cytokinesis in Animal Cells" (Cambridge University Press, Cambridge, UK, '96). He describes the historical context of his work and the work of his contemporaries. Others used electron microscopy, fluorescent antibodies, microinjection of inhibitory antibodies and genetic knockout to show that a contractile ring composed of actin filaments and myosin-II generates the force to constrict the furrow. Nevertheless, progress on cytokinesis has been less spectacular than in many other areas of cell biology. From my point of view, progress has been modest for several reasons. First and foremost, cytokinesis has not been amenable to a reductionist biochemical attack because the process requires the complex environment of a live cell. Additional impediments include a very large inventory of participating molecules and the ephemeral nature of a structure that appears, carries out its function and disassembles in a few minutes, a small fraction of a single cell cycle.

Ray's experiments established an agenda that continues to challenge students of cytokinesis. This is a major accomplishment, made all the more remarkable by a distinctly 19th century approach. Open questions include the identity of the molecule(s) that carry the temporal and spatial information required for cytokinesis from the mitotic apparatus to the cortex, the mechanism that carries these molecules to the cortex, the cortical receptors for these signals, mechanism that assembles the contractile ring, the signal that triggers the contraction of the ring and the mechanism that disassembles the ring as it contracts. Given the complexity of the system, much of this information will have to emerge from studies on genetically tractable systems such as yeast, flies and nematode worms. One hopes that evolution has been sufficiently parsimonious that the molecules identified in these systems can be placed in the spatial and temporal context defined by Ray Rappaport in echinoderm eggs.