JCB Meeting Report

Rappaport Furrows on Our Minds: The ASCB Cytokinesis Meeting Burlington, VT July 22–25, 2004

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Some of the basic principles of cytokinesis were established 40 or more years ago. Cytokinesis researchers since then have struggled with the confusions inherent in redundant and interdependent pathways. Now, however, enough molecular detail is accumulating to bring some order to thoughts on how one cell becomes two.

"*When I began working on cytokinesis, I thought I was tinkering with a beautifully made Swiss watch, but what I was really working on was an old Maine fishing boat engine: overbuilt, inefficient, never-failed, and repaired by simple measures.*" – Ray Rappaport (keynote address)

Cytokinesis presents us with two mysteries: how a cleavage furrow is localized to a single, defined place in the cell; and how an accumulation of proteins can deform a perfectly stable ovoid shape to create two new cells (Fig. 1). Scientists working in varied organisms met recently in Burlington, VT, to address these questions.

Each question has a simple answer, although those answers turn out to be only a start. Ray Rappaport, the godfather of cytokinesis, began the meeting with a wonderful chalk-talk keynote address that tackled the first question. In describing cytokinesis research over the past 100 years he mentioned a good deal of his own work, which established that the mitotic apparatus determines furrow placement. Rappaport and his frequent co-author Barbara Rappaport used sand-dollar eggs to test different theories. (There were no new theories, he said: "By 1903, all the possible physical mechanisms that I could imagine…had been proposed.") The eggs had the advantage of persisting in the face of incredible abuse: they kept dividing after being turned into donuts, squeezed into tubes, and poked with needles. Through these manipulations Rappaport found that wherever the mitotic apparatus went, so followed the cleavage furrow.

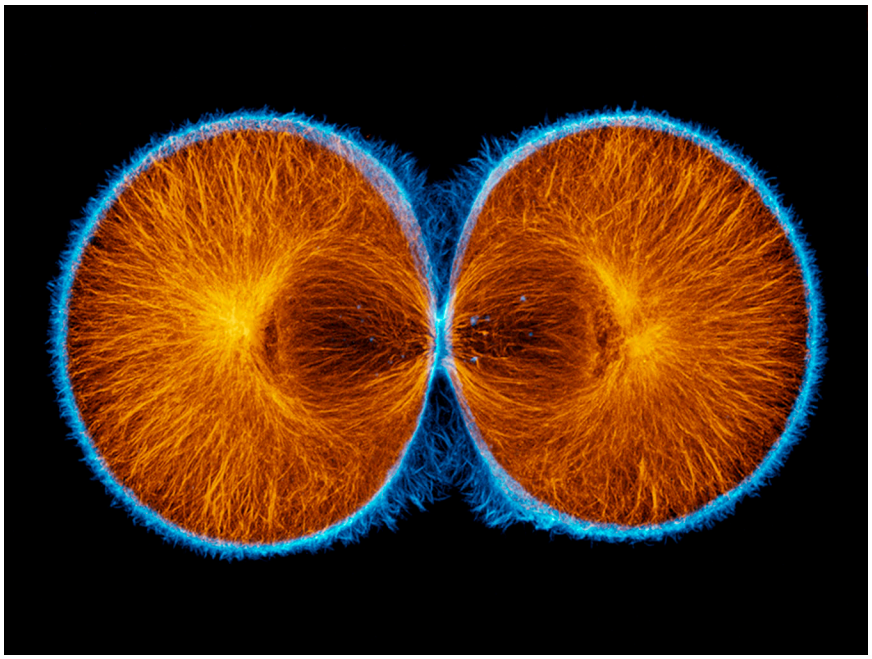

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Figure 1. **Cytokinesis, here seen in the green urchin, requires both microtubules (orange) and actin (blue).**

Once in place, the furrow constricts—a force that was directly measured by Rappaport (1967). The leading theory for the underlying mechanism remains the purse-string model of actomyosin contraction, but the number of exceptions and add-ons is threatening to bury this model in a mountain of caveats.

As predicted by Rappaport's quote, the meeting proved that cytokinesis is packed with work-arounds and redundant pathways. The pathway that appears critical in one organism may be vestigial in another, but participants seemed to embrace the idea that underlying mechanisms are shared, and in the different model systems it is often only the relative emphasis that varies.

Positioning the cytokinetic apparatus: who's on first!

One of the things that may differ considerably between organisms is the rela-

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tive timing of different cytokinesis events, so the description of a single, definitive sequence may be impossible. Nevertheless, Jian-Qiu Wu and Tom Pollard (Yale University, New Haven, CT) have tackled the task for fission yeast. Wu recorded the order of assembly using fluorescently tagged proteins and found that proteins arrived at the furrow in the following sequence: the anillin-like protein Mid1p; Myosin II and myosin light chains; IQGAP; the PCH protein Cdc15p and the formin Cdc12p; F-actin; tropomyosin; α-actinin; capping protein; an unconventional myosin II; and then lastly the septins (Wu et al., 2003).

The early arrival of Mid1p (and, in other organisms, of its rather distant relative anillin) invites speculation that anillin is the mythical furrow inducer dubbed stimulin. This remains possible in fission yeast, where Mid1p arrives at the furrow via export from the nucleus. Mid1p's early arrival was confirmed by Rafael Daga and Fred Chang (Columbia University, New York, NY), who performed an intriguing variation on Rappaport's sand dollar manipulation experiments (Rappaport, 1985). When Daga and Chang changed the position of the yeast nucleus by gentle centrifugation before anaphase onset, cortical Mid1p and the site of future cytokinesis followed the nucleus, and moving the nucleus could even induce multiple furrows. However, if the nucleus was moved after anaphase onset, the contractile apparatus did not relocalize, presumably because it was locked in place and unlinked from the nucleus.

Fission yeast that are mutant for *mid1* are highly defective in furrow placement, so at least in this organism this protein has all the characteristics of a functional marker of furrow positioning. In other model systems, however, anillin has not fulfilled its early promise. It does interact with septins, myosin, and actin, but it is either not essential or only essential for late events, and often arrives later than or at the same time as other furrow components. Chris Field (Harvard Medical School, Boston, MA) thinks anillin is probably a structural component that helps attach the contracting cortex to the plasma membrane. So perhaps anillin is not stimulin after all. "It's too bad," said David Burgess (Boston College, Chestnut Hill, MA). "I think we were all wishing it were."

The many possible actions of microtubules

Furrow positioning by the fission yeast nucleus is a special case, because most other eukaryotic cells lack a nuclear envelope during the lead-up to cytokinesis. Instead they have a mitotic apparatus composed of a spindle and two microtubule asters. The overlap between two asters can induce a furrow at the cortex, even when the asters are positioned back-to-back in a cell with two spindles (Rappaport, 1961). The search for the nature of this aster-initiated stimulus is still continuing more than forty years after Rappaport's classic experiment.

There are now two mysteries about microtubules during cytokinesis: what they are doing to the cortex; and how they are doing more (or less) of it at the furrow versus the rest of the cortex. At the meeting there was evidence for both of the two major models of furrow induction: astral relaxation (astral microtubules dampen down cortical contraction everywhere except the furrow); and equatorial stimulation (special microtubules near the furrow provide a pro-contraction signal) (Glotzer, 2004). Michael Glotzer (IMP, Vienna, Austria) emphasizes that all the models may contribute. "This process is far more complicated than we expected," he said, "and there are probably multiple ways to generate a furrow."

Relax! This is not the furrow

Evidence supporting the astral relaxation model—the idea that microtubules may suppress contractility—came from Ted Salmon (University of North Carolina, Chapel Hill, NC) and Bruce Bowerman (University of Oregon, Eugene, OR). Both saw ectopic furrowing (or what appeared to be furrows) when microtubules were destroyed with either nocodazole (Canman et al., 2000) or an excess of a microtubule-severing protein that is normally down-regulated after meiosis (Kurz et al., 2002). The caveat, of course, is that wholesale depolymerization may be releasing any number of furrow-promoting or furrow-interfering mediators from anywhere along the microtubules. Ted Hinchcliffe (University of Notre Dame, IN) also reported ectopic furrowing in cell fragments (karyoplasts) lacking centrosomes.

Glotzer suggested one possible model to explain how astral relaxation microtubules might be kept away from the furrow. He suggested that the cell creates a local minimum of microtubule density at the cell equator by both the separation of centrosomes and the bundling of midzone microtubules into a central spindle during spindle formation. Only when both pathways are compromised does cytokinesis fail in worms (Dechant and Glotzer, 2003).

The idea of negative regulation outside of the furrow also gained tentative support from an observation by Bill Bement (University of Wisconsin, Madison, WI) and George von Dassow (Friday Harbor Laboratories, Friday Harbor, WA). Their main achievement was to visualize a tight band of activated RhoA in the cleavage furrow of sea urchin eggs using a labeled Rho-binding domain. (Activated Rho can stimulate actomyosin contractility via several mechanisms.) Localization was microtubule but not actin dependent. In the absence of actin (and, more occasionally, in the presence of actin), lightning bolts of active RhoA were seen shooting toward the spindle poles. Bement suggested that these bolts might represent active RhoA being picked off the non-furrow membrane by dynamic microtubules, and that furrow-localized RhoA might be protected from such action by the stability of microtubules in this area.

Direct equatorial stimulation

Such stable, furrow-localized microtubules could contribute to an equatorial stimulation mechanism. The existence of these microtubules has been reported by Julie Canman and Ted Salmon (Canman et al., 2003), as discussed at the meeting by Salmon (Uni-

versity of North Carolina, Chapel Hill, NC). Canman and Salmon looked at both monopolar and bipolar spindles, and reported finding a subpopulation of stable microtubules that extended past chromosomes before binding to the cell cortex at the site of furrow formation. In the monopolar version of these experiments, the furrow always formed on the side of the spindle containing the chromosomes. This suggests that chromosomes may supply microtubules with factors that promote both microtubule stability and furrowing. Katie Shannon and Salmon also reported that the site where taxol-stabilized microtubules contacted the cortex correlated with the site of furrowing.

Another tantalizing clue about the origin of different types of microtubules came from Fumio Motegi, working with Asako Sugimoto (Center for Developmental Biology, Kobe, Japan). Motegi used RNAi to show that centrosomal nucleation of microtubules is dependent on the presence of either γ-tubulin or Aurora-A kinase. When only γ-tubulin was present microtubules near the cortex fluctuated in length and furrowing was stimulated, but when only Aurora-A was present microtubules grew persistently and furrowing was suppressed. How these findings translate to the arrangement of possibly heterogeneous microtubules in wild-type spindles is not yet clear.

Pat Wadsworth (University of Massachusetts, Amherst, MA) described one possible source of dynamic polar microtubules. She reported that microtubule release from centrosomes increased sevenfold upon anaphase onset. Microtubules were transported toward the polar not equatorial cortex and were associated with components of the centrosome such as pericentrin.

Having furrow-localized microtubules that are "different" is all very well, but the next step is to work out what those microtubules are doing or delivering. Some clues are emerging. Burgess has focused on a previously reported interaction between EB1, which localizes to microtubule ends, and the dynein partner dynactin. He sees this complex preferentially stabilized near the equatorial cortex, with the dynactin also translocating to the furrow itself. In the furrow it is associated with lipid rafts that move to the furrow and apparently serve as a localized source of signaling by Src-family kinases. This signaling is required for cytokinesis, and may lead to Ca^{2+} release that activates myosin light chain kinase (MLCK) and thus myosin activity. Some logical and molecular gaps remain in this pathway, but the sighting of rafts in fission yeast furrows by Chang provides exciting corroboration.

Burgess believes that these events occur very early, and indeed Karen Lee and Andrew Miller (Hong Kong University of Science and Technology, Hong Kong) showed that a Ca^{2+} transient precedes the appearance of the cleavage furrow in zebrafish embryos (with two other transients appearing later; Lee et al., 2003). They also suggested that the probable source of the Ca^{2+} transients—a localization of ER and IP_3 receptors that they observe on either side of the furrow—may get there thanks to microtubule-based transport.

Overlap as initiator

As a variant of the equatorial stimulation model, a special signal could come from antiparallel, interdigitating microtubules. These microtubules originate from the two centrosomes of the spindle or from within the spindle and then meet either in a defined "central spindle" or in more subtle appositions of antiparallel microtubules. Kinesin motors such as Mklp1 may get trapped in such areas if they walk outwards on one microtubule only to fall off and walk back on an antiparallel microtubule. There is an expanding cast of characters in this region, including the interacting proteins Mklp1 (also known as ZEN-4, Pavarotti, and CHO1), a Rho family GAP (known variously as CYK-4, MgcRacGAP, or RacGAP50C) and a Rho family GEF (known as ECT2 or Pebble). The microtubule-binding protein Orbit/MAST/CLASP, the microtubule-bundling protein PRC1 (and homologues Ase1p in budding yeast, MAP-65 in plants, and SPD-1 in worms) and perhaps the chromosomal passenger proteins may also contribute to establishment or maintenance of central spindle structure or function. Chromosomal passenger proteins, for example, get to normal and ectopic cortical furrow sites before myosin does (Eckley et al., 1997).

The central spindle has been thought of as driving late events in cytokinesis, as in most systems disruption of central spindle formation does not inhibit furrow initiation but rather results in furrow regression. Indeed, the presence of a GAP may help in disassembly of the actomyosin ring as contraction winds down. But the presence of the Rho-activating GEF suggests that central spindle components may also induce furrow formation (Somers and Saint, 2003). In some organisms the central spindle is large and comes close to the cortex; in others the central spindle may be too distant from the cortex but the relevant structures may be more subtle microtubule overlaps that are closer to the cortex.

Sometimes the distinction between the central spindle and chromosome-dependent versions of the equatorial stimulation model may be subtle. Factors that in one cell type get to the furrow via specially stabilized astral microtubules that pass by chromosomes may in other cell types get there by binding to regions of microtubule overlap. The first mechanism would explain why Canman and Salmon see furrow formation on the chromosome side of monopolar spindles, and the second mechanism would explain both the localization of some chromosomal passenger proteins to chromosome-free regions of microtubule overlap and the induction of furrow formation by such regions (Rappaport, 1961; Savoian et al., 1999). The situation is further complicated by hints that passenger and central spindle proteins may target to central spindle and furrow cortex using independent pathways within a single cell type (Murata-Hori and Wang, 2002; Verbrugghe and White, 2004).

Let the squeezing begin

Things really get going when actin and myosin show up. Issei Mabuchi (University of Tokyo, Japan) showed that in

fission yeast the COOH-terminal 134 residues of myosin II are necessary and sufficient for myosin's accumulation at the division site, and that phosphorylation of a serine within that region prevents premature localization (Motegi et al., 2004). This mechanism is dependent on Mid1p, the distant relative of anillin. Once again, the relevance to organisms that lack a nuclear envelope during parts of mitosis is unclear.

For those other organisms, somehow all the microtubule action must translate into formation and activation of an actomyosin ring. In one parallel with Mabuchi's results, Jim Spudich (Stanford University, Stanford, CA) showed that both F-actin and the entire NH_2-terminal half of myosin II (containing all of the actin-activated ATPase activity and light chain binding regions) are dispensable for furrow accumulation of myosin II in *Dictyostelium*. Instead, a kinesin may be involved.

Once myosin is localized, its activation could occur downstream of either a Ca^{2+} signal (which would turn on MLCK; see above) or activated Rho (itself turned on by Pebble on the central spindle). Activated Rho would presumably turn on myosin by first activating Rho kinase (ROCK), which also targets myosin light chain.

Fumio Matsumura (Rutgers University, Piscataway, NJ) described how the activation of myosin could be restricted to the correct phase of the cell cycle. He has found that myosin phosphatase targeting subunit (MYPT) is phosphorylated by Cdc2 kinase during mitosis. This ensures that Polo-like kinase (PLK) binds to MYPT until the end of mitosis, but with the decline of Cdc2 activity the PLK falls off, allowing ROCK to deliver an inactivating phosphorylation. This frees myosin from the negative regulation by MYPT. Several groups also reported that citron, another kinase targeting myosin, adds yet another layer of regulation.

Actin is thought to be dragged in primarily via the actions of myosin contractility. But the formins, which promote actin polymerization, may also get into the act. The Src family kinases mentioned by Burgess are known to bind to formins, and binding of formins by active Rho can release the formins' auto-inhibition.

Once actin and myosin are present and active, the cell starts to contract into two. Yu-li Wang (University of Massachusetts Medical School, Worcester, MA) was an early proponent of the purse-string model for this contraction, but in his talk he indicated that reality may be more complicated. His group has locally disrupted actin organization via targeted cytochalasin D. Drug application in the furrow region accelerated constriction, presumably by speeding up disassembly of the shrinking actomyosin ring or by softening up the cytoplasmic gel that must be squeezed out of the way of the furrow (O'Connell et al., 2001).

Myosin II appears to be involved in this disassembly process during normal cytokinesis, as blebbistatin, a myosin II inhibitor that interferes with cytokinesis (Straight et al., 2003), suppresses actin turnover. Wang suspects that blebbistatin may inhibit cytokinesis by both stabilization of the cortex and relaxation of forces.

This view of the cell as a single elastic entity is consistent with that proposed by Doug Robinson (Johns Hopkins, Baltimore, MD). He stated that cytokinesis occurs at a rate much slower than would be predicted by a simple contractile ring mechanism, and that therefore counteracting forces must be active to inhibit the rate of furrowing. A mutant analysis in *Dictyostelium* showed that RacE and the actin cross-linker dynacortin (Girard et al., 2004) act together to slow down cytokinesis. Dynacortin is enriched in polar regions of the cell, underlining the idea that F-actin regulation outside of the furrow is probably critical for successful cytokinesis.

In Robinson's view, the cell must initially deform away from its equilibrium shape, struggling against the cortical viscoelasticity. But once it deforms far enough, these same forces drive final furrow thinning as the cell strives once again to minimize its surface area-to-volume ratio.

Finishing things off: abscission

Despite all of the effort a cell makes to position and constrict the contractile ring, the job is not done until the two daughter cells separate from each other during the final stage of cytokinesis: abscission. There are microtubules and actomyosin rings to get out of the way, and one membrane must be converted into two without allowing any leakage.

This process seems to involve the microtubule-rich midbody (the final product of the central spindle) within the intercellular bridge. Rytis Prekeris (University of Colorado Health Science Center, Denver, CO) reported on how the proteins Rab11-FIP3 and Rab11-FIP4, which are known to localize to the midbody (Horgan et al., 2004), get to their destination and bring in recycling endosomes. The Prekeris group found that calcium induces binding of Rab11-FIP3 to ARF6 GTPase and thus recruitment to the midbody. Rab11-containing recycling endosomes are then targeted to the midbody via binding to Rab11-FIP3. The Prekeris group has found that this system is essential for the completion of cytokinesis.

Just how early this system is brought into play may depend on the organism and cell type. A fly homologue of the FIP proteins called nuclear fallout (Nuf) is associated with recycling endosomes and required early for proper actin deposition at the pseudocleavage furrows of syncytial embryos, according to Blake Riggs and Bill Sullivan (University of California, Santa Cruz, CA) (Riggs et al., 2003). These structures invaginate rather than constrict around an existing membrane, so they may have a greater need to recruit new membrane. Sullivan noted that recycling endosomes, which are connected to centrosomes, may be uniquely suited for positioning at the furrows.

Steve Doxsey (University of Massachusetts Medical School, Worcester, MA) and Arnaud Echard (working with Patrick O'Farrell at the University of California, San Francisco, CA) have found proteins that may help secretory vesicles or endosomes to fuse with the furrow. Echard took part in an RNAi-based screen for fly cells that failed in cytokinesis and found that α-SNAP, a protein involved in recycling membrane fusion proteins, is essential for

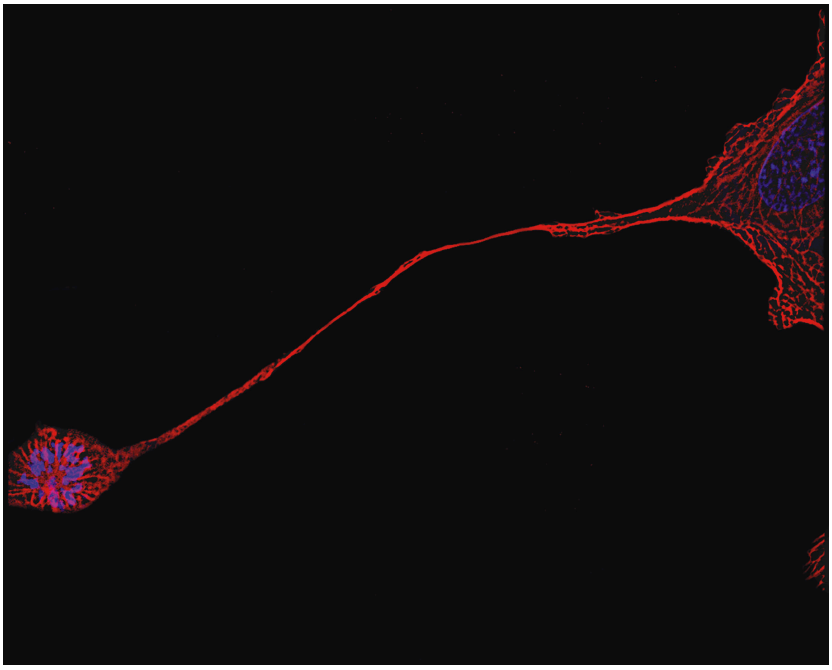

Figure 2. **Cells lacking centriolin make it through most of cytokinesis but remain interconnected by long intercellular bridges.**

maintaining the stability of the intercellular bridge (Echard et al., 2004). Doxsey started with a centriole-binding protein called centriolin that later moves to the midbody (Gromley et al., 2003). He now reports that centriolin binds to both exocyst (vesicle targeting) and SNARE (vesicle fusion) proteins; centriolin binding is required for their localization to the midbody. RNAi against these proteins mimics the phenotype after loss of centriolin (Fig. 2): cytokinesis fails at a late stage and cells start to lose their midbody entirely or make extremely elongated and persistent intercellular bridges.

Back to the boat engine

For almost every result presented at this conference there was a piece of contradictory information. For example, after all the talk of the importance of the central spindle there were the data from Koen Verbrugghe and John White (University of Wisconsin, Madison, WI). They found that cytokinesis proceeded in worm embryos partially inactivated for SPD-1 function even though these embryos never formed a central spindle. There was still some deposition of central spindle proteins such as ZEN-4 at the cortex, however, suggesting that there may be another pathway by which these proteins can reach the cortex (Verbrugghe and White, 2004). Meanwhile data from Dahong Zhang (Oregon State University, Corvallis, OR) conflict with the idea that chromosomes are needed for furrow positioning. He used micromanipulation in meiotic grasshopper spermatocytes to show that microtubule bundles were the minimum requirement for furrow onset in these cells (Alsop and Zhang, 2003).

John Pringle (University of North Carolina, Chapel Hill, NC) emphasized that proteins such as septins and even myosin are sometimes but not always essential, depending on the organism. It seems that different systems can compensate for each other: if actomyosin constriction is compromised then membrane addition comes to the rescue; and in budding yeast the machinery that builds the septum can stand in for other cytokinesis processes.

Amy Maddox and Karen Oegema (University of California, San Diego, CA) reported that at least two redundant pathways exist to promote furrow ingression in worm embryos—the first involving anillin upstream of septins, and the second involving Rho kinase. When either pathway was removed, furrow completion was slightly delayed but cytokinesis was successful. However, when both pathways were compromised, cytokinesis was dramatically slowed and frequently failed.

Such redundancy may partly explain why several exhaustive screens for new components have, according to talks at the meeting, turned up mostly familiar friends (Skop et al., 2004). And the repeated findings of redundancy are affecting how the community interprets its results. "We have to be less dogmatic," says Glotzer. "An experiment that proves that something is not required in one system does not necessarily indicate that is it not required in any system."

Although this meeting highlighted the fundamental knowledge we have gained about cytokinesis, several key questions remain. First to mind is the ever-elusive stimulin, the hypothetical molecule that acts to position the furrow. So far, anillin is the first known marker at the furrow, but is there another factor that gets there even earlier? Is stimulin a multiprotein complex or could it be a nonproteinacious factor, such as Ca^{2+} ions or components of the membrane itself? Second, what is the role of actin dynamics in cytokinesis? Several labs doing cytokinesis screens have turned up molecules such as Arp2/3 and Cdc42, which are typically thought to modulate the rapid turnover of actin, which is seemingly incompatible with a simple purse-string model. Third, when and how do cells complete cytokinesis? Is membrane fusion the vehicle that drives cell separation? And lastly, a need for community-wide unification of the nomenclature—both for proteins and microtubule subsets active during cytokinesis—was addressed at this meeting. There is currently no uniform nomenclature and the literature is becoming chaotic.

After this meeting we all understood a little more about cytokinesis, but the innate redundancy and multiple steps of regulation will continue to make understanding the "Maine boat engine" that we call cytokinesis a very challenging task. As Jim Spudich stated in the closing talk, "there's a lot of wonderful work being done, but boy do we have a long way to go!"

Thanks to keynote speaker, Ray Rappaport, organizer, Yu-li Wang, and the co-organizers, Christine Field, Tom Pollard, Bruce Bowerman, and David Burgess. Additional thanks to Amy Maddox, Bruce Bowerman, David Kovar, Katie Shannon, William Bement, Michael Glotzer, Christine Field, and individual authors for comments on this manuscript.

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