Brinkley-Fest of Mitosis

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"Mitosis: Spindle Assembly and Function," a conference in honor of Dr. Bill R. Brinkley, brought together many researchers to discuss progress in the field and celebrate the many contributions that Dr. Brinkley has made.

A FASEB meeting in honor of Dr. Bill Brinkley, organized by Conly Rieder and Bob Palazzo, was held near Palm Springs in June 2007. The hot topic, fueled in part by the fiery desert venue, was all things mitosis. The 55 speakers and 57 posters covered subjects ranging from the G2/M and metaphase/anaphase transitions to kinetochore/centrosome function, spindle assembly, chromosome segregation, cytokinesis, and microtubule pharmacology—many areas in which Brinkley has made major contributions over the past 50 years. Below I describe some of the work presented and highlight recent advances.

Kinases, Phosphatases, and Mitotic Progression

The key upstream regulator of mitosis (Figure 1) is cyclin-dependent kinase 1 (Cdk1), in complex with its activator cyclin B, whose synthesis is required for mitotic entry, and destruction for mitotic exit. Jon Pines highlighted the importance of localization of cell cycle regulators for proper mitotic progression. By following the disappearance of GFP-tagged cyclins in cultured cells using time-lapse fluorescence microscopy, he saw that targeting cyclin B to the centromeres of chromosomes preserved proper timing of its destruction, while localizing it to the plasma membrane or spindle poles delayed its degradation. Interestingly, the *a*naphase *p*romoting *c*omplex or *c*yclosome (APC/C), which marks substrates for destruction by modifying them with ubiquitin, is also found on chromosomes, suggesting that a key strategy for degradation of mitotic regulators is to colocalize them with the mediators of their destruction. However, the APC/C is also found at spindle poles, and Peter Jackson described a mechanism by which anchoring APC/C at the poles in a complex with Emi1, NuMA, and dynein (the END network) actually prevents premature cyclin B degradation (Ban et al., 2007). Therefore, localization on the spindle can provide either positive or negative regulation of cyclin B stability and exit from mitosis.

Mitotic induction by Cdk1 requires not only cyclin B accumulation but also the removal of an inhibitory phosphate group, which is mediated by Cdc25 phosphatase, which in turn functions in opposition to Wee1 kinase. Protein localization is also a key regulatory mechanism at this step, when nuclear accumulation of both Cdk1/cyclin B and Cdc25 tip the balance toward Cdk1 dephosphorylation and activation (Hutchins and Clarke, 2004).

The opposing Wee1 kinase itself is an active topic of investigation, and Tin Tin Su described experiments in *Drosophila* embryos showing that Wee1 has additional, Cdk1-independent roles in regulating microtubule growth and organization during spindle assembly by interacting with and likely phosphorylating other targets, including the gamma tubulin ring complex that promotes microtubule nucleation (Stumpff et al., 2005), and the mitotic kinesin-5, which promotes spindle bipolarity.

Another kinase that has an important impact on mitotic entry is the p38 stress-activated kinase. Conly Rieder showed that when human cells are exposed to stresses like microtubule inhibitors, osmotic shock, or DNA damaging agents during the G2 phase of the cell cycle, p38 is activated and mitotic entry is delayed, an effect that can be mimicked by adding the p38 activator Anisomycin. Rieder surveyed isogenic cell lines to evaluate the efficacy of this arrest pathway at different stages of cancerous transformation, using long-term, time-lapse phase microscopy that allowed him to measure mitotic durations. Although p38 could be activated in transformed cells, its ability to arrest the cells decreased with increasing degrees of transformation, indicating a checkpoint bypass that fosters oncogenesis. Greenfield (Kip) Sluder spoke about another pathway of cell cycle arrest that depends on p38 but operates in G1. This pathway can be activated by a variety of insults, including cell microsurgery, laser ablation, or even exposure to blue light. Thus, stresses that would not impact the cell cycle on their own can act additively to activate a p38-dependent arrest in G1.

Numerous kinases, including the Polo and Aurora kinase families, are crucial for progression through mitosis. Distinguishing among the multiple mitotic roles of a single kinase has been difficult because standard approaches, like RNA interference, are not rapid enough, and kinase disruption can have indirect effects leading to cell cycle arrest through checkpoint activation. Prasad Jallepalli described an approach to circumvent these problems and study Polo-like kinase 1 (Plk1) function in late mitosis. Using gene knockout techniques in human tissue culture cells, endogenous Plk1 was replaced with a mutant version that could be drug-inhibited rapidly, specifically, and with great temporal precision. This chemical genetics approach revealed a role for Plk1 to promote cleavage furrow formation by targeting RhoA GTPase to
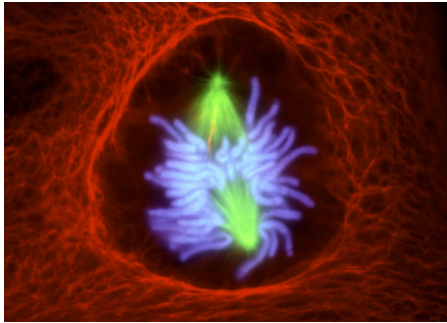
Figure 1. A Newt Lung Cell in Mitosis
Fluorescence micrograph of a newt lung cell in mitosis showing the mitotic spindle stained for microtubules (green) and chromosomes (blue). Keratin is in red. Image taken by Conly Rieder.

the equatorial cortex during anaphase (Burkard et al., 2007). Aurora A and B kinases are well known to play roles in spindle microtubule organization and chromosome segregation. In addition, Karen Oegema described a microtubule-independent role for Aurora A in regulating the timing of nuclear envelope breakdown (Portier et al., 2007). The much less well-characterized Aurora C kinase is a current topic of investigation in the Brinkley lab, as reported by Rebecca MacCorkle. Aurora C is expressed only in mammals, primarily in developing gametes. Their experiments indicate that it can operate in pathways similar to those of Aurora B, interacting with chromosomal passenger protein complex members as well as the centromeric histone CENP-A, thereby regulating chromosome congression and segregation during mitosis and meiosis.

Centriole Assembly and Duplication

One cellular structure that continues to fascinate is the centrosome, an extremely important microtubule organizing center (MTOC), which in vertebrate cells consists of a pair of centrioles surrounded by amorphous pericentriolar material (PCM), where microtubule nucleation takes place (Doxsey et al., 2005). The centrosome plays key roles in cell division, making centrosome assembly, duplication, and function active areas of investigation. Centrosome duplication begins during S-phase when new daughter centrioles grow orthogonally to the existing ones. Tim Stearns showed how centrosome duplication is "licensed" for the next cell cycle by disengagement of the two centrioles during mitotic exit (Tsou and Stearns, 2006). Interestingly, centriolar disengagement is regulated by Separase, the same protease that promotes sister chromatid separation at the onset of anaphase. It will be of great interest to determine (1) whether the centrosomal function of Separase requires its protease activity, and (2) the identity of its downstream targets.

To ask whether centriolar duplication requires a specific template site on the mother centriole, Alexey Khodjakov demonstrated that brute force can be applied with great precision. He used a laser to ablate a newly forming

daughter centriole in S-phase arrested cells and found that another daughter subsequently forms on the mother, but not necessarily at the same site. Their model is that the mother does not provide a defined template for centriole assembly, but rather organizes the PCM to provide a permissive environment for daughter formation. In his talk, Jordan Raff described his lab's work, which shows that the *Drosophila* centriolar protein Sas-4 is required for centriole replication. Mutant fly embryos lacking Sas-4, which is essential for the rapid early divisions that require centrosomes, develop normally thanks to maternally supplied protein. Sas-4 mutant adults completely lack centrioles and centrosomes, but remarkably, they are morphologically quite normal. However, these flies die soon after birth because they also lack sensory cilia essential for them to eat (Basto et al., 2006).

Another approach to get at the interesting question of how centrioles assemble has been taken by Alexander Dammermann and Karen Oegema, who have developed a quantitative fluorescence-based assay to examine the process in the early *C. elegans* embryo. Dammermann described his results from using embryos expressing GFP-tagged SAS-6 and SAS-4, proteins required for sequential steps in centriole assembly. By elucidating the dynamics of these key centriolar components in living embryos, this approach provides an important step toward a molecular understanding of centriole duplication.

Multiple Mechanisms of Spindle Assembly

Much of the current research on spindle assembly aims to elucidate the multiple mechanisms that function in parallel to promote formation of the bipolar microtubule array (Kline-Smith and Walczak, 2004; Wittmann et al., 2001). By acting as microtubule nucleation centers and defining the spindle poles, duplicated centrosomes constitute an important cue in most cell types. Chromosomes also promote spindle assembly by providing microtubule capture sites at their kinetochores and biochemical activities through chromatin-localized enzymes such as RCC1, the guanine exchange factor that generates a chromosome-centered gradient of RanGTP (Goodman and Zheng, 2006). RanGTP controls multiple essential functions during mitosis by releasing spindle assembly factors (SAFs) from importin nuclear transport receptors, thereby promoting microtubule nucleation and organization around chromatin (Gruss and Vernos, 2004).

Distinguishing the relative contribution of centrosome- and chromatin-mediated mechanisms in cells harboring both elements has been a challenge. Pat Wadsworth described her lab's creative approach using mitotic mammalian cells treated with nocodazole to depolymerize spindle microtubules and subjected to an incomplete nocodazole washout, thereby slowing microtubule regrowth and allowing better visualization of different microtubule populations. Inhibition of the Ran-regulated SAF TPX2 impaired microtubule polymerization at chromosomes, but not at centrosomes, which could still capture chromosomes and move them rapidly poleward due to activity of the microtubule minus-end-directed motor

cytoplasmic dynein (Yang et al., 2007). Interestingly, although chromosomes were captured, kinetochore fibers never formed in the absence of TPX2 (Tulu et al., 2006), suggesting that both centrosomal and chromatin pathways play essential roles in proper spindle assembly. Claire Walczak discussed experiments elucidating the function of another minus-end-directed spindle motor, the human kinesin-14 HSET. Their model is that RanGTP regulates the ability of HSET to cross-link kinetochore fibers to spindle microtubules, which impacts both spindle length and kinetochore fiber function.

Another Ran-regulated SAF is Maskin, a member of the transforming acidic coiled-coil (TACC) family of microtubule-associated proteins studied by Christine Wiese. Wiese described biochemical assays to dissect Maskin's functions at the centrosome, showing that Maskin is not required for microtubule nucleation at reconstituted centrosomes, but without Maskin, these centrosomes display a higher rate of microtubule release. Maskin/TACC plays multiple roles in centrosome function and spindle assembly (O'Brien et al., 2005), and the Wiese lab has shown that Ran regulates the phosphorylation of Maskin by Aurora A kinase (Albee et al., 2006). An important goal is to determine whether and how Ran regulates other Maskin activities.

RanGTP is not the only signal generated by chromosomes. Hiro Funabiki spoke about a different chromatin pathway of spindle assembly mediated by Aurora B, a component of the chromosomal passenger complex (CPC), which includes INCENP, Survivin, and Dasra A/B (Gassmann et al., 2004; Sampath et al., 2004). Hiro's lab has shown that phosphorylation of Aurora B substrates, such as histone H3 and stathmin, is suppressed by phosphatases in the cytoplasm, but CPC binding to chromatin induces substrate phosphorylation (Kelly et al., 2007). Interestingly, microtubules can also induce substrate phosphorylation. Since the Ran pathway promotes microtubule stabilization, it may create a positive feedback loop by promoting CPC pathway activation. A feature characteristic of the CPC is its translocation from the segregating chromosomes to the spindle midzone during anaphase (Vader et al., 2006). Tarun Kapoor described *f*luorescence *r*esonance *e*nergy *t*ransfer (FRET)-based sensors to monitor Aurora B kinase activity in living cells. Together, experiments addressing the molecular mechanisms and dynamic localization of CPC activity are providing new insights into the role of this complex in spindle assembly and function.

Specifying Centromeres

An essential element of chromosome inheritance is the centromere, which defines the site of kinetochore formation on each sister chromatid, allowing their attachment to the spindle and accurate segregation to daughter cells. Centromere assembly in most species, with the exception of budding yeast, is not specified by DNA sequence, but by an epigenetic mark. The basis of this mark is thought to be a histone H3 variant called centromere protein A (CENP-A), which assembles into centromeric nucleo-

somes. Bill Brinkley played a key role in elucidating centromere specification by helping to characterize human autoantisera that by immunofluorescence analysis recognized antigens present at centromere regions (Brenner et al., 1981). These sera were later used by Bill Earnshaw to identify CENP-A, CENP-B, and CENP-C (Earnshaw and Rothfield, 1985; Earnshaw et al., 1987). This opened up the field by facilitating molecular characterization of kinetochores, and the two Bills have generously provided these reagents to many researchers in the field. Furthermore, the Brinkley lab was the first to show that overexpression of CENP-A could recruit other kinetochore proteins to ectopic sites on chromosomes (Van Hooser et al., 2001), which supported the notion that CENP-A nucleosomes act not only as a mark but also as the structural foundation for kinetochore assembly.

Recently, major progress has been made toward understanding how and when CENP-A is deposited at centromeres during the cell division cycle, and the mechanisms by which CENP-A chromatin directs assembly of the kinetochore-spindle microtubule interface. Don Cleveland described his lab's work identifying the *CENP-A* *c*entromere *t*argeting *d*omain (CATD), which can confer centromere-specific nucleosomal properties to histone H3, including the ability to rescue the depletion of endogenous CENP-A (Black et al., 2004, 2007). Using a variety of cleverly tagged CENP-A variants, the Cleveland lab identified a group of novel proteins associated with CENP-A nucleosomes (Foltz et al., 2006). They also showed that new CENP-A incorporation is temporally restricted to early G1 and requires exit from mitosis (Jansen et al., 2007). A complementary approach to investigate kinetochore specification has been taken in Arshad Desai's lab, using RNA interference-based screens in *C. elegans* to identify factors that result in a "kinetochore-null" (KNL) phenotype, first defined by inhibition of CENP-A. Desai discussed KNL-2, a Myb-DNA binding domain-containing protein that is required for incorporation of CENP-A into centromeric histones, and whose localization to centromeres is restricted to the time of new CENP-A incorporation (Maddox et al., 2007). Also through use of affinity tags, the Desai lab has identified a large group of interacting kinetochore proteins that span all the way from centromeric chromatin to the microtubule interface (Cheeseman et al., 2006). Together, these and similar studies recently published by the group of Mitsuhiro Yanagida (Fujita et al., 2007) advance our understanding of centromeric chromatin assembly and provide a framework for investigating the molecular mechanisms of higher-order kinetochore assembly (Carroll and Straight, 2007).

CENP-A is clearly necessary for centromere assembly and function, but what is sufficient to specify a centromere? The Keynote Lecture by Bill Earnshaw addressed this interesting question. Bill and his collaborators Hiroshi Masumoto and Vladimir Larionov have created a faithfully transmitted human artificial chromosome (HAC) using a DNA array based on a repeated alpha satellite sequence containing a CENP-B binding motif and tet-operators, to which proteins can be targeted by transfection of

tet-repressor fusions. Interestingly, the HAC, which also recruits CENP-A and CENP-C, was strongly destabilized by targeting of a transcriptional transactivator that promotes heterochromatin formation. These experiments highlight that the epigenetic centromere is sensitive to chromatin conformation. The opportunity to target different proteins into an active centromere provides a novel approach to investigate both mechanistic and structural aspects of the kinetochore.

Kinetochore-Microtubule Attachments

To understand how the kinetochore-microtubule interface is built up from the centromere, a critical question is where the ~50 known kinetochore proteins localize relative to one another. Ted Salmon described a fluorescence imaging technique, *k*inetochore-speckle *h*igh-*r*esolution *c*olocalization (K-SHREC), which can locate protein epitopes along the axis of sister kinetochores at metaphase to an accuracy of ~15 nm. The relative positions of CENP-A and several distal kinetochore components involved with microtubule attachment, including the Ndc80 complex, the Mis 12 complex, and Knl1, have already been determined (Kotwaliwale and Biggins, 2006; Wei et al., 2007). This approach promises to provide novel information about the dynamic mechanical behavior of kinetochore proteins under different conditions of microtubule attachment and tension. The rate of protein turnover at kinetochores is also an important issue. Jagesh Shah described methods using *f*luorescence *r*ecovery *a*fter *p*hotobleaching (FRAP) and *f*luorescence *c*orrelation *s*pectroscopy (FCS) to study how checkpoint proteins at the kinetochore could transduce signals to the cytoplasm to inhibit the APC and prevent anaphase onset when kinetochore-microtubule attachments are absent or incorrect (Shah et al., 2004; Wang et al., 2006). Current efforts are focused on a novel checkpoint protein, p31comet, and dynamics measurements have led to a computational model supporting the hypothesis that an unattached kinetochore both activates an APC inhibitor and inhibits an APC activator.

The depletion of many different varieties of kinetochore proteins leads to defects in spindle microtubule attachment. An ongoing challenge is to distinguish which of these are required to build the kinetochore structure in the first place, which ones directly mediate microtubule attachments, and which ones function to correct improper attachments by regulating microtubule-kinetochore interactions (Maiato et al., 2004). Tim Yen described a novel mammalian protein called Tripin/Sgo2 that appears to fall into the third class. Tripin-depleted cells displayed chromosome attachment defects resulting in lagging chromosomes at anaphase. The underlying cause is thought to be the mislocalization of the microtubule-destabilizing protein MCAK, which is implicated as part of an elaborate error-correction system that eliminates improper microtubule-kinetochore connections so that correct end-on attachments can be made (Huang et al., 2007). Another exciting development in the field has been the discovery of a set of kinetochore proteins that

leads a double life, localizing to the nuclear pore during interphase. Mary Dasso discussed this class of nucleoporins that moonlights in mitosis, including the nine-protein Nup107-160 complex, which is kinetochore associated throughout mitosis, and RanGAP/RanB2/Ubc6(sumo ligase), which requires microtubules, as well as RanGTP and the exportin CRM1, for its delivery to kinetochores (Arnaoutov et al., 2005). Interestingly, altering levels of RanGTP inhibits kinetochore function (Arnaoutov and Dasso, 2003), and RanGTP/CRM1 function is required for proper kinetochore fiber formation and microtubule attachment. These studies demonstrate that, in addition to its role in spindle assembly, the Ran pathway plays important roles at the kinetochore that compel further investigation.

Kinetochores and Chromosome Movement

Organized chromosome movement depends on the interaction of dynamic spindle microtubule fibers (K-fibers) with the kinetochores of each sister chromatid, and structural studies are crucial to understanding the basis of this interaction. Bill Brinkley was among the first to investigate kinetochore structure by electron microscopy, and his classic work (Brinkley and Stubblefield, 1966) has spawned many generations of subsequent studies. This field is still very active, and the Palm Springs conference included several presentations exploring the structural and mechanical features of the kinetochore-microtubule interface. In vertebrates, the critical juncture between microtubule plus-ends and kinetochores occurs at the outer plate, a 50 nm thick disk-shaped structure. Bruce McEwen described his study of this plate by electron tomography of PtK1 cells, which revealed a network of cross-linked 10 nm diameter fibers in the absence of microtubules. The fibers became shorter upon microtubule attachment, with some fibers forming a radial mesh around microtubule ends, and other fibers extending out to attach to microtubule walls (Dong et al., 2007). These observations support a model in which the outer plate functions as a flexible network that rearranges to form multiple, low-affinity attachments to each microtubule (Cheeseman et al., 2006). The network model differs from the repeat subunit model first proposed by Bill Brinkley's lab in that microtubule-binding components are distributed throughout a network rather than organized into multiple discrete microtubule binding sites. Nevertheless, electron tomography also provides evidence that the outer plate network is organized into multiple, loosely connected patches. These patches could correspond to the linear array of CREST-containing subunits observed when centromeric DNA is stretched (Zinkowski et al., 1991). This modified version of the repeat subunit model retains the strong appeal of accounting for the structural and evolutionary diversity of kinetochores and centromeres among eukaryotic chromosomes of many species.

An exciting development has been the identification in budding yeast cells of a mechanistically plausible coupler that could simultaneously ensure stable spindle fiber

attachment to chromosomes and permit microtubule shortening. The Dam1/DASH complex, a heterodecameric kinetochore protein assembly with a strong affinity for microtubules, can polymerize into rings around microtubules in vitro (Miranda et al., 2005; Westermann et al., 2005), and was discussed by Julie Welburn from the Nogales lab. Rings around kinetochore microtubules have not yet been seen by electron microscopy of yeast cells, but the coupling properties of rings may have significant advantages. A ring whose inner diameter is larger than that of the microtubule wall, and one that binds tubulin with strong, flexible links, is capable of transducing a large fraction of a microtubule's conformational energy while maintaining a firm grip on its depolymerizing end. These features appear to be particularly well suited for budding yeast, where each kinetochore is stably attached to only one microtubule. The Dam1/DASH complex has not yet been found in organisms other than yeasts, and even in fission yeast, its components are not essential for mitosis (Sanchez-Perez et al., 2005). Thus, other structures are more likely to be the physiologically significant couplers in most mitotic spindles.

Several microtubule-dependent motor enzymes, including dynein and two or three kinesins, have previously been identified as components of the kinetochore, so much of the field has focused on the role of motors in chromosome-to-pole motion. The talk by Richard McIntosh, however, cast this problem in a different light. He summarized work from his lab showing ATP-independent chromosome motion in vitro, suggesting that microtubule depolymerization can drive anaphase-like movements (Lombillo et al., 1995); then, he sketched more recent work on mutant fission yeasts. Deletion of the genes encoding all of this cell's pole-directed motor enzymes had no effect on the maximal speed of poleward chromosome motion, suggesting that other factors can drive these movements in vivo (Grishchuk and McIntosh, 2006). His group is now studying structures that bind microtubules to kinetochores and that might transduce the energy stored in microtubules into the work necessary for mitotic motions. Using electron tomography to examine the structure of the kinetochore-microtubule interface in mammalian cells, the McIntosh lab has observed slender fibrils that run from centromeric chromatin to the tips of microtubules, where individual protofilaments are splaying outward as a manifestation of microtubule dynamics. The connection to bending protofilaments suggests that these fibrils may be mechanical couplers between microtubule dynamics and chromosome motion, and the structural similarity between the kinetochore fibrils and the phylogenetically conserved Ndc80 kinetochore protein complex (Wei et al., 2007) carries the hopeful possibility that this kind of coupling mechanism will be found in mitotic structures from a wide range of organisms.

Although microtubule-kinetochore coupling mechanisms may be motor independent, a number of motor proteins function to position mitotic chromosomes in animal cells at least in part by stimulating microtubule dynamics. Jason Stumpff from Linda Wordeman's lab described

their study of Kif18A, a kinesin-8 motor. This interesting class of kinesins can translocate to and subsequently disassemble the plus-ends of microtubules. High-resolution live cell imaging and kinetochore tracking experiments revealed that Kif18A overexpression suppresses prometaphase chromosome oscillations, while its depletion promotes chromosome motility and oscillation amplitude, suggesting that Kif18A functions to increase the switching rate between poleward and antipoleward chromosome movement while decreasing the velocity of chromosome movement.

Anaphase and Cytokinesis

The ultimate goal of chromosome attachment and movement by spindle microtubules is to segregate the sister chromatids to opposite spindle poles so that the cytokinetic furrow can divide the cell into two daughters, each with a full complement of chromosomes. During anaphase A in higher eukaryotes, chromosomes move poleward by two mechanisms linked to microtubule depolymerization. Kinetochores actively depolymerize attached microtubule plus-ends, a behavior termed "Pacman" (after the Namco video game Pac-Man), and are reeled into the spindle poles by a continual poleward movement of the microtubule lattice driven by minus-end depolymerization, called flux. David Sharp's lab has been exploring the roles of microtubule-depolymerizing kinesins that promote anaphase A chromosome movements at the kinetochore and the spindle pole in *Drosophila* embryos and S2 cells (Rogers et al., 2004). Their latest experiments have identified another depolymerase, Klp59D, which contributes to the rate of microtubule depolymerization at both sites. Sharp also described evidence for roles of three different microtubule-severing enzymes. Spastin and Fidgetin localize to the centrosome, where they could potentially excise gamma tubulin ring complexes from microtubule minus-ends, while Katanin is present on the anaphase chromosomes, where it may stimulate microtubule depolymerization and Pacman chromosome motility (Zhang et al., 2007). Helder Maiato then described an additional possible role for microtubule flux. When poleward microtubule flux in *Drosophila* S2 cells is blocked, anaphase poleward chromosome movement becomes highly asynchronous, which could lead to chromosome missegregation. Thus, flux may promote mitotic fidelity by providing a uniform distribution of spindle forces (tension) that controls and coordinates chromosome segregation.

Anaphase B, the process of spindle pole separation, also plays an important role in the physical relocation of sister chromatids to daughter cells. Jonathan Scholey gave a historical perspective of his lab's work in *Drosophila* embryos using biochemistry, microscopy, and computational modeling approaches to learn how a kinesin-5-driven sliding filament mechanism cooperates with antagonistic motors and microtubule dynamics to generate a balance of forces defining steady-state spindle pole position during metaphase and anaphase A. In response to cyclin B degradation at anaphase B onset, this balance is tipped so that the spindle elongates. Based

on their analysis of microtubule turnover before and just following anaphase B initiation, the Scholey lab proposes that spindle pole separation occurs upon inhibition of microtubule depolymerization at the spindle poles, allowing outwardly sliding interpolar microtubules to drive pole-pole separation. Modeling suggests that this would depend on the establishment of a spatial gradient of microtubule plus-end catastrophe frequencies which decrease toward the equator (Cheerambathur et al., 2007).

How the cytokinetic furrow is accurately positioned midway between the spindle poles has been a topic of long-standing investigation and debate (Glotzer, 2004), and two talks at the meeting dealt specifically with this issue. Dahong Zhang described the sophisticated methods by which his lab uses micromanipulation of insect spermatocytes to move spindle components around and monitor the distribution of contractile actin filaments. They have found that microtubule asters exclude cortical actin filaments, while overlapping plus-ends of central spindle microtubules stimulate the formation of cortical actin patches. These results indicate that two mechanisms previously proposed as alternatives, polar relaxation and equatorial stimulation, actually cooperate to position the division plane. Examination of diverse organisms can help to reveal conserved players in the process. Fred Chang described genetic and cytological approaches to dissecting division plane positioning in fission yeast, in which contractile ring position is determined by the location of the nucleus prior to mitosis. Chang's lab has identified the anillin-like protein, mid1p, as one important factor. It is a peripheral membrane protein that recruits other contractile ring elements, but there are also both positive and negative regulators of mid1p placement. One of the negative regulators is pom1 kinase, which is localized to the cell poles indirectly by microtubules. Thus, convergent findings in both animal cells and yeasts indicate that positive equatorial signals and negative signals from the poles contribute to proper placement of the division plane, and understanding the molecular basis of these signals is now coming within our grasp.

Aneuploidy and Checkpoints

Failures in chromosome segregation lead to aneuploidy, a hallmark of cancer. However, the relationship between aneuploidy and cancer, and the exact mechanisms by which cancer cells become aneuploid, are poorly defined. Two very interesting talks dealt with this topic. Duane Compton presented a cell biological study of aneuploidy in which he investigated the cause of chromosome missegregation using live cell imaging to directly examine mitosis in chromosomally stable and unstable cell lines, and found a high incidence of lagging chromosomes during tumor cell anaphase. Artificially increasing lagging chromosomes with drug treatments increased the rate of chromosome missegregation as measured by FISH, and could induce chromosomal instability in normal cell lines. A noteworthy finding of this study is that increasing chromosome missegregation alone was not sufficient to convert diploid cells into highly aneuploid cells, indicating that other phenotypic changes are required to generate the extreme ploidy changes characteristic of malignant cells. Beth Weaver has identified the kinetochore kinesin CENP-E as one factor crucial to maintenance of the diploid state, and described experiments using CENP-E mutant mice to investigate the effects of aneuploidy, since CENP-E heterozygosity causes whole-chromosome aneuploidy in the absence of other defects, such as structural rearrangements of the chromosomes or elevated levels of DNA damage. Interestingly, while Weaver found an increase in spontaneous spleen and lung tumors in aged animals, aneuploidy appeared to suppress tumor formation in the context of a pre-existing chromosomal instability, supporting the hypothesis that moderate rates of chromosome loss drive tumorigenesis while high levels promote cell death (Weaver et al., 2007).

To avoid chromosome segregation defects and aneuploidy, cells have sophisticated surveillance mechanisms to monitor whether two sister kinetochores are bound to microtubules from opposite spindle poles. In addition to mediating chromosome attachments and movements, the kinetochore also serves as a platform for assembly of checkpoint complexes that halt progression to anaphase in response to chromosome attachment defects. However, one type of defect, called a merotelic attachment, in which one sister kinetochore is connected to microtubules from both spindle poles, does not activate the canonical checkpoint. Daniela Cimini discovered that merotelic attachments occur frequently in early prometaphase, and are a leading cause of aneuploidy (Cimini et al., 2001, 2003). In her presentation, Cimini described recently published results showing that Aurora B kinase plays a critical role in preanaphase correction of merotelic attachments by promoting microtubule turnover at the kinetochore (Cimini et al., 2006). She also described current experiments indicating that Aurora A kinase contributes to correction of merotelic attachments as well. The next challenge is to determine whether the two Auroras function through common or distinct pathways.

Several presentations addressed the functions of specific proteins involved directly or indirectly in the kinetochore-based checkpoint. Stephen Taylor described his lab's use of a conditional allele of the core checkpoint protein Bub1 to investigate its function in mice. Similar to results from other checkpoint gene knockouts, Bub1 is essential for early embryogenesis, but the use of a conditional approach has allowed them to inactivate Bub1 at later stages of embryogenesis and in adult tissues, revealing that Bub1 is also required for organogenesis and spermatogenesis. Based on experiments in cultured mouse embryo fibroblasts, their working model is that Bub1 not only prevents anaphase onset in the presence of unaligned chromosomes, but also promotes metaphase chromosome positioning. Gordon Chan spoke about dynein-associated protein ZW10, which appears to play a supporting role in checkpoint function by affecting the targeting and dynamics of core components (Karess, 2005). The Chan lab is undertaking extensive structure-function analysis of ZW10 to identify its functional domains, and

Figure 2. Bill Brinkley "Roast" Dinner Table
Left to right, standing: Bob Palazzo, Bill Brinkley, Kevin Brinkley, Don Cleveland, and Ted Salmon; seated: Bill Earnshaw, Dick McIntosh, Conly Rieder, and Tim Hunt.

has found that interaction with hZwint-1 is required for stable hZW10 binding to prometaphase kinetochores and checkpoint function, and that the Rod-ZW10-Zwint complex responds to the loss of kinetochore tension by accumulating at those kinetochores in an Aurora B kinase-dependent manner.

In his talk, Bill Sullivan described an altogether different kind of chromosome-mediated checkpoint, in which DNA damage induced by site-directed endonuclease cleavage can delay the metaphase-anaphase transition, dependent on the Grp/Chk1 kinase (Royou et al., 2005). Interestingly, acentric fragments induced by the cleavage recruit BubR1 and Polo and move to the poles during anaphase, leading to the model that the metaphase delay provides time for the chromosome fragments to generate ectopic kinetochores that allow them to be cleared from the metaphase plate, thereby preventing them from interfering with cytokinesis.

Perspectives

All at the Palm Springs meeting (selected participants are pictured in Figure 2) would likely agree that it is an exciting time to be studying mitosis. Many in the large cast of characters playing regulatory and mechanical roles in cell division have been identified, and we are now at the fascinating stage of mapping networks and figuring out exactly how they function. The increasing sophistication and beauty of imaging techniques, and the development of chemical and computational tools, have revolutionized the field. One notable theme was that multiple mitotic mechanisms function in parallel to ensure fidelity of the process and are utilized to differing degrees among organisms. This is great news, because it means that researchers working in diverse systems can make important contributions, and none of us should be out of work for some time (funding issues aside!). Above all, the meeting reminded us that Bill Brinkley is a pioneer and role model, with regard to both his contributions to our understanding of mitosis and his tireless advocacy of research.

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