

## The Cell Center at 100

## Meeting Review

Tim Stearns\* and Mark Winey†

\*Department of Biological Sciences  
Stanford University

Stanford, California 94305-5020

†Department of Molecular, Cellular and  
Developmental Biology

University of Colorado, Boulder

Boulder, Colorado 80309-0347

The centrosome was among the first organelles described by early cell biologists at the end of the 19th century. A recent meeting of investigators working on the microtubule organizing center thus marked a century of progress on understanding the organelle that is at the center of cell organization and division ("Centrosomes and Spindle Pole Bodies," 1997, ASCB/EMBO/H. Dudley Wright Conference). The meeting, the first of its kind in this field, was nucleated by the organizers, John Kilmartin (MRC, Cambridge, U.K.) and Robert Palazzo (University of Kansas, Lawrence, Kansas), expressly to bring together investigators working on the two most prominent microtubule organizing center model systems: the yeast spindle pole body (SPB) and the animal cell centrosome.

In any discussion of microtubule organizing centers, it is best to first define terms, as there is much confusion, even among the cognoscenti, about the meaning of many of the terms used in this field. E. B. Wilson wrote in his classic 1925 cell biology text *The Cell in Development and Heredity* (Wilson, 1925) that "centrosomes are easier to define in physiological terms, than in morphological terms." These were prophetic words, as the diverse morphologies of centrosomes and SPBs still cannot be reconciled, but their function is largely the same. Both organelles nucleate the polymerization of microtubules from free tubulin subunits and organize those microtubules into arrays that are used for specific purposes in interphase, mitosis, and meiosis. In addition, both centrosomes and spindle pole bodies duplicate once per cell cycle so that there are always only one or two per cell, depending on the stage of the cell cycle.

Boveri was the first to use the terms centriole and centrosome, in his remarkable work at the turn of the century (reviewed in Wilson, 1925). A modern definition of these would be that the centriole is a short cylinder of nine triplet microtubules and that the centrosome is a pair of centrioles surrounded by pericentriolar material (see Figure 1). It is this pericentriolar material that is responsible for microtubule nucleation. The role of the centrioles in the centrosome is still unknown, but it is clear that they can initiate the growth of a cilium under appropriate conditions. A centriole at the base of a cilium is called a basal body. The known morphological diversity of microtubule-organizing organelles expanded greatly with the advent of electron microscopy and the identification of the SPB in fungal cells. The SPB is a disc-shaped structure embedded in the nuclear envelope that organizes nuclear microtubules from one face

and cytoplasmic microtubules from the other. In contrast to the centrosome, SPBs lack centrioles and consist of several distinct layers. The goal of modern research on the centrosome/SPB is to understand the molecular underpinnings of the conserved functions of nucleation, organization, and duplication, and to more clearly define the function of the organizing center in microtubule-dependent processes.

#### Microtubule Nucleation and $\gamma$ -Tubulin

The discovery of  $\gamma$ -tubulin by Oakley and Oakley (1989), and the subsequent demonstration that  $\gamma$ -tubulin is conserved in all eukaryotes, is localized to the centrosome/SPB, and is required for microtubule nucleation (reviewed in Joshi, 1994), led to a simple model for microtubule nucleation. In this model,  $\gamma$ -tubulin is a special tubulin tethered to the centrosome/SPB, where it aids in the initiation of the complex microtubule lattice structure by acting as a template for the addition of  $\alpha$ - and

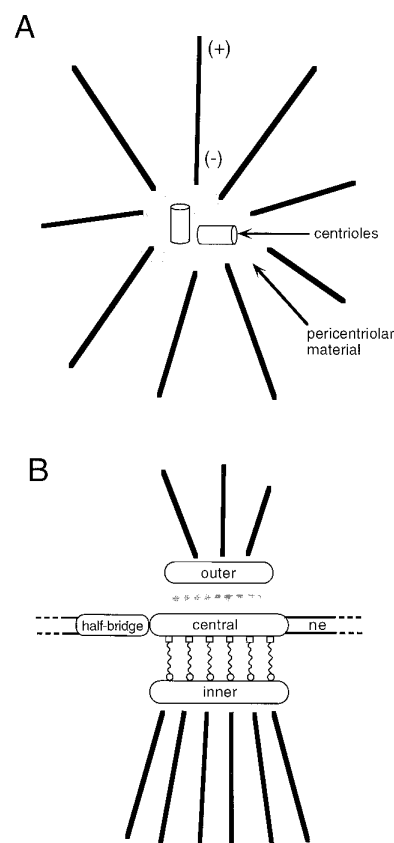


Figure 1. Schematic Representation of a Centrosome and a Spindle Pole Body

The centrosome is about 1  $\mu\text{m}$  in diameter, and the SPB is about 100 nm in diameter. Microtubules, shown as black lines, are nucleated from the pericentriolar material of centrosomes (A) and from the inner and outer plaques of the SPB (B). The centrosome is often associated with, but physically separate from, the nucleus, whereas the central plaque of the SPB is embedded in the nuclear envelope (ne). The half-bridge structure of the SPB is the site of initiation of a new SPB.

$\beta$ -tubulin subunits. The  $\gamma$ -tubulin nucleator model was supported strongly by the discovery that  $\gamma$ -tubulin exists in the cytoplasm as part of a large complex (Stearns and Kirschner, 1994). This complex has the shape of an open ring of about the same diameter as a microtubule (Zheng et al., 1995) and is found at the ends of microtubules at the centrosome (Moritz et al., 1995). Originally purified from *Xenopus* egg cytoplasm, it is now clear that the  $\gamma$ -tubulin complex is conserved in animals. Y. Zheng (Carnegie Institution, Baltimore, Maryland) and M. Moritz (UCSF, San Francisco, California) reported the purification of the complex from *Drosophila* embryos, and T. Stearns (Stanford University, Stanford, California) reported the purification of the complex from mammalian cells. The *Drosophila* complex is also ring-shaped, and the protein composition of the fly, frog, and mammalian complexes is similar.

E. Schiebel (Max Planck, Martinsried, Germany) reported that in the yeast *S. cerevisiae*, there is also a  $\gamma$ -tubulin complex, but that it is much smaller than the animal cell complex, containing only  $\gamma$ -tubulin and two proteins previously identified as interacting with  $\gamma$ -tubulin in yeast, Spc97p and Spc98p (Geissler et al., 1996; Knop et al., 1997). Interestingly, fractionation of the large  $\gamma$ -tubulin complex from *Drosophila* embryos yields a smaller subcomplex that consists of  $\gamma$ -tubulin and two other proteins (M. Moritz, Y. Zheng). One of these proteins is a sequence homolog of Spc98p, and there is evidence that the other is a homolog of Spc97p (T. Stearns, Y. Zheng). Thus, the components of the  $\gamma$ -tubulin complex that are most closely associated with  $\gamma$ -tubulin are conserved and likely to be playing similar functional roles.

Although the hypothesis that  $\gamma$ -tubulin is the microtubule nucleating agent of the centrosome remains unchallenged, the relationship between the ring complex and nucleation is hotly debated. The yeast  $\gamma$ -tubulin complex described by E. Schiebel is too small to be a ring, and K. Oegema (UCSF, San Francisco, California) and M. Moritz reported that the small  $\gamma$ -tubulin subcomplex from *Drosophila* does not form a ring, yet is capable of nucleating microtubule assembly. There have been two models proposed for microtubule nucleation, one in which the  $\gamma$ -tubulin ring complex acts as a template, directing the formation of a microtubule with the same dimensions as the ring (Zheng et al., 1995), the other in which the open ring actually represents the resting state of a linear  $\gamma$ -tubulin polymer that can interact laterally with tubulin subunits to nucleate assembly (Erickson and Stoffer, 1996). Although the microscopic evidence favors the first model, the results from yeast and flies mandate a closer examination of the actual nucleation mechanism. This is a problem that will likely require reconstitution experiments with purified components.

In addition to the very specific experiments on  $\gamma$ -tubulin and its immediate neighbors in the centrosome, there were several reports on the identification in yeast of genes that interact with  $\gamma$ -tubulin, but do not appear to be components of the  $\gamma$ -tubulin complex (L. Marschall, Stanford University, Stanford, California; K. Siegers, Max Planck, Martinsried, Germany; J. Vogel, Yale University, New Haven, Connecticut). The assembly

and regulation of nucleating structures at the centrosome/SPB is likely to be complex, and it will be a big job to sort out the function of all the genes involved.

#### **The Organizing Center Parts List**

Centrins are a family of small calcium-binding proteins that are similar in structure to calmodulin and, like  $\gamma$ -tubulin, are conserved components of microtubule organizing centers (reviewed in Salisbury, 1995). The family includes members from yeast, plants, and animals, all closely related to each other. Centrin was first identified as a component of calcium-modulated contractile fibers in green algae (Salisbury et al., 1984), and the yeast homolog, Cdc31p, was identified as a mutant with a defect in SPB duplication (Baum et al., 1986). Little is known about exactly what centrin does at the centrosome/SPB. Results presented at the meeting suggest that centrin, like calmodulin, interacts with several partners, some with functions not related to organizing center function. M. Rose (Princeton University, Princeton, New Jersey) started on the theme of diverse function, reporting the identification of a new yeast kinase, *KIC1*, that is associated with Cdc31p in cells and is dependent on Cdc31p for activity. Conditional mutations in *KIC1* exhibit defects in cellular morphogenesis, but not SPB duplication. This, and several other lines of genetic evidence, suggest that both Cdc31p and Kic1p perform multiple functions.

In mammalian cells there is the additional complexity of multiple centrin proteins. M. Bornens (Institut Marie Curie, Paris, France) reported the isolation of a new member of the centrin family in humans, HsCEN3, bringing the number of human genes to three (Middendorp et al., 1997). This new centrin more closely resembles the yeast Cdc31p, suggesting that it might play a role in centrosome duplication. Functional analyses of this protein in yeast (S. Middendorp and M. Bornens, Institut Marie Curie, Paris, France) and of a *Xenopus* centrin, Xcen, reported by L. Urbani (Stanford University, Stanford, California) and T. Stearns, suggest roles in centrosome duplication or function, although the phenotypes are somewhat pleiotropic, consistent with several functions. The issue of dual function for organizing center components was recognized early on in the study of calmodulin in yeast (Geiser et al., 1993). Calmodulin is not only an SPB component, but is also found at sites of cell growth. This sort of result is likely to become more frequent as components of the regulatory network controlling organizing center function and duplication are uncovered. Such components are likely to be common to several structures or pathways in the cell.

Despite the obvious complexity of the organelles, only a few components are known to be conserved between centrosomes and SPBs:  $\gamma$ -tubulin, spc97, spc98, and centrin. It is not yet clear how far the analogy between the centrosome and SPB can be taken in terms of protein homology—the building blocks of these very different structures are likely also to be different at a fundamental level. Still, there is much more work to be done in identifying components before such a question can even be considered. Accordingly, much of the research presented was directed toward generating the organizing center parts list. An emerging theme was that many of the newly identified proteins have predicted coiled-coil domains as their predominant structural feature.

A particularly important talk was given by J. Kilmartin. In collaboration with O. Jensen and M. Mann (EMBL, Heidelberg, Germany), Kilmartin has completed a "first pass" analysis of the make-up of SPBs using MALDI (matrix assisted laser desorption ionization) mass spectrometry. Peptide mixtures derived from gel-isolated proteins found in an SPB preparation were mass analyzed. Because the sequence of all yeast genes is known, assigning sets of peptide masses to an ORF by database searching is straightforward (Shevchenko et al., 1996). Approximately 50 proteins were identified, many previously known components of the spindle and SPB and at least 5 new ones, each of which was shown independently to localize to the SPB. The amazing power of this approach led some (not Kilmartin!) to suggest that more traditional methods of identifying genes in yeast, such as by genetic interactions with known components, were now obsolete. In fact, the MALDI technique failed (probably for trivial technical reasons) to identify  $\gamma$ -tubulin in the SPB; thus, it seems likely that a combined genetic and biochemical approach will still be necessary to identify all of the components of the SPB. Indeed, M. Snyder (Yale University, New Haven, Connecticut) reported the identification of an SPB component via a genomic screening technique.

An exciting report from S. Dutcher (University of Colorado, Boulder, Colorado) concerned the finding that the *UNI3* locus of *Chlamydomonas* encodes a new tubulin that clearly is not an  $\alpha$ -,  $\beta$ -, or  $\gamma$ -tubulin. *UNI3* is not essential for viability, but mutant cells lose their flagella and display anomalies in their basal bodies. The Uni3 protein is localized to the basal bodies, but not to the spindle poles. There is no equivalent protein in the yeast genome, but yeast cells lack any structure corresponding to the basal body/centriole. There are sequence homologs in the mammalian EST databases, suggesting that this new tubulin will be a conserved centriole component.

Several presenters reported on new components of the centrosome. R. Palazzo discussed the use of clam oocyte extracts to produce and to enrich for large amounts of centrosomal material. Antibodies made against this material have been used to identify at least two new proteins, one of which, CEP135, is conserved in vertebrates and appears to be a component of pericentriolar material (R. Kuriyama, University of Minnesota, Minneapolis, Minnesota). M. Bornens reported the further characterization of ninein, previously shown also to be a coiled-coil component of pericentriolar material (Bouckson-Castaing et al., 1996). When either CEP135 or ninein is overexpressed in cells, the proteins accumulate in the pericentriolar material, and in the case of CEP135 extraordinary filamentous structures are observed. The isolation and characterization of such aberrant structures, as has been done with overexpression of yeast SPB components (Kilmartin and Goh, 1996; Sundberg et al., 1996), may be informative as to the binding partners and structural characterization of the components.

The components discussed above are likely to be important for the structure and function of the microtubule organizing center. It is important to note, however, that localization to the organizing center alone is not a

sufficient criterion to establish functional importance, as there are now several examples of proteins that seem to use the centrosome/SPB as a "landing pad," having nothing to do with organizing center function per se. Two prominent examples came from studies in the fission yeast, *S. pombe*. I. Hagan (University of Manchester, Manchester, U.K.) reported experiments undertaken in collaboration with M. Sohrmann and V. Simanis (ISERC, Lausanne, Switzerland) that showed the Cdc7 protein kinase, essential for septum formation, localized first to both SPBs, and later in cycle to only one of the two SPBs. The difference between the poles appears to be in the activation state of a Sgi1p, a GTP-binding protein that is at both poles, but in the GDP-bound form at the pole without Cdc7p, and the GTP-bound form at the pole with Cdc7p. C. Sparks and D. McCollum (Worcester Foundation, Shrewsbury, Massachusetts) showed that another protein kinase required for septation, Sid2p, is present on the SPB throughout the cycle, but also appears at the actin ring that precedes the cleavage furrow at telophase. D. Glover (University of Dundee, Dundee, U.K.) pointed out the connection between SPB separation and cytokinesis revealed by disrupting the fission yeast gene *pol1*, which encodes a homolog of polo kinase essential for both processes (Ohkura et al., 1995). Glover also described defects in both cytokinesis and polo kinase localization resulting from loss of function of a *Drosophila* kinesin-like protein.

It is not yet clear whether the organizing center participates actively or passively in functions such as those above that do not appear to involve microtubule function. A possible rationale for using the organizing center as a cytoplasmic assembly point is that localization to such a restricted area of cytoplasm would greatly increase the effective concentration of proteins, facilitating important protein-protein interactions. Given the results from the simple yeast cell, it is a safe bet that the situation in animal cells will be more complicated and that functional tests will be required to distinguish the necessary components from the freeloaders.

Lastly, several speakers addressed the issue of how the components of the organizing center are held together, with the experimental approach being to blow them apart. Spectacular disintegration of the organizing center was reported to be caused by injection of antibodies against centriolar tubulin (M. Bornens), by overexpressing regulatory molecules (A. Fry and E. Nigg, University of Geneva, Switzerland), or by mutations in SPB components (T. Davis, University of Washington, Seattle, Washington). Splitting of spindle poles was observed in cells overexpressing dynamitin, the 50 kDa subunit of dynactin, thus implicating cytoplasmic dynein activity in pole organization (C. Echeverri and R. Vallee, Worcester Foundation, Shrewsbury, Massachusetts). These results raised the question of how dynamic the centrosome and SPB are: can they be thought of as solid-state objects in the cytoplasm, or are they constantly changing by accretion and loss of components? The latter seems most likely, at least for the centrosome, as it is already clear that the organelle can change dramatically in differentiated cell types. Many of the proteins that hold the organizing center together have coiled-coil motifs. Lest one think that such proteins will

simply be boring struts in a larger assembly, consider that for Spc110p, one such coiled-coil SPB component, mutations in different functional domains exhibit diverse mutant phenotypes (T. Davis). Spc110p is known to bind calmodulin (Geiser et al., 1993) and was shown to interact with the Tub4p/Spc97p/Spc98p complex (E. Schiebel). If the complexity of Spc110p is any indication, dissecting the various functions of individual pole components will be quite interesting.

#### ***Seeing the Organizing Center in a New Light***

The remarkable architecture of the centriole was first revealed by thin-section EM, but by the same technique, the pericentriolar material is nondescript in comparison. More advanced types of analysis are now revealing the true complexity of the organizing center. E. Bullitt (Boston University School of Medicine, Boston, Massachusetts) found that one of the layers at the center of isolated *S. cerevisiae* SPBs consists of a paracrystalline hexagonal array of Spc42p (Bullitt et al., 1997), a major coiled-coil protein component of the SPB. Overexpression of Spc42p results in dramatic two-dimensional growth of the crystalline array in the plane of the nuclear envelope (Donaldson and Kilmartin, 1996). In normal cells, the lateral dimension of the SPB is correlated with ploidy, 2n cells have a larger SPB than 1n cells. Bullitt indicated that there appears to be a protein boundary surrounding the Spc42p array in the SPB, suggesting that this could be involved in regulating the size of the organelle.

Another approach is to image the entire organizing center using electron tomography, a technique in which the sample is viewed in the EM from many angles, allowing a three-dimensional reconstruction after computer processing. Both Moritz et al. (1995), working with purified *Drosophila* centrosomes, and R. Palazzo (Vogel et al., 1997), working with purified clam centrosomes in collaboration with C. L. Rieder (Wadsworth Center, Albany, New York), showed results in which rings could be discerned in the previously featureless pericentriolar material. These rings are about 25 nm in diameter and are assumed to represent centrosome-bound  $\gamma$ -tubulin ring complexes. This raises an important issue about microtubule nucleation: if the  $\gamma$ -tubulin ring complexes are the same at the centrosome and in solution, then why does nucleation appear to be limited to the centrosome? Among the most intriguing possible answers is that the  $\gamma$ -tubulin complex is able to nucleate microtubule polymerization equally well in the cytoplasm and in solution, but that the concentration of  $\gamma$ -tubulin at the centrosome and the pole-forming influences of certain microtubule motors result in an array centered on the centrosome.

Standard EM of serial thin-sections was used to describe the SPB duplication cycle in *S. pombe* (J. R. McIntosh, University of Colorado, Boulder, Colorado; Ding et al., 1997). Unlike *S. cerevisiae* where the SPB is embedded in the nuclear envelope through the entire cell cycle, the *S. pombe* SPB shuttles in and out of the nuclear envelope. In interphase the SPB is on the cytoplasmic side of the nuclear envelope; prior to the initiation of mitosis, a "fenestra" in the nuclear envelope opens and the SPB enters and nucleates the microtubules of the mitotic spindle. The nuclear envelope appeared to be intact during interphase, making this SPB

a completely cytoplasmic organelle like the centrosome. The difference with *S. cerevisiae* may be more semantic than actual however, as McIntosh also presented evidence that  $\gamma$ -tubulin is concentrated on the nuclear side of the envelope at the position of the SPB, indicating that there must be communication through the envelope during interphase. I. Hagan in collaboration with the Boulder Lab for 3D Fine Structure demonstrated that the Cut12 protein is localized to the nuclear envelope-proximal side of the *S. pombe* SPB during interphase. These talks reminded all that the diversity of organizing center morphologies and functional strategies extends far beyond the animal centrosome and the *S. cerevisiae* SPB, and that we would be wise to keep an eye out for useful exceptional cases.

At the light microscopic level, S. Doxsey (University of Massachusetts Medical School, Worcester, Massachusetts) reported the application of deconvolution and immunofluorescence techniques to the centrosome with fascinating results. Deconvolution refers to the removal of out-of-focus information from a set of microscope images using a computer algorithm. Doxsey has shown that  $\gamma$ -tubulin and pericentrin (Doxsey et al., 1994), two components of the pericentriolar material, are found together in a beautiful lattice structure around the centrioles. The colocalization of  $\gamma$ -tubulin and pericentrin seems to have functional significance as well, in that overexpressed pericentrin forms structures in the cytoplasm that recruit  $\gamma$ -tubulin and nucleate microtubules, suggesting that  $\gamma$ -tubulin ring complexes may be organized on a structural lattice of pericentrin. Using the same technology, R. Kuriyama found a distinct lattice of CEP135 in the pericentriolar material.

#### ***Miraculous Duplication***

The organizing center must duplicate each cell cycle so that each of the cells resulting from mitosis has one organizing center. The several claims of an organizing center-specific nucleic acid have now been discredited; thus, we are left without any simple explanation for the "once and only once" duplication of the microtubule organizing center during a normal cell cycle. Two distinct approaches to solving this problem were represented at the meeting: an organizing center-based approach, in which components and regulators are examined for changes in activity, localization, or modification during the duplication process, and a cell cycle-based approach, in which the global regulators of the cycle are examined for an effect on duplication. On the components side, K. Gull (University of Manchester, Manchester, U.K.) reported that maturation of a newly duplicated centriole was correlated with the addition of the protein cenexin. This maturation event involves structural modification of the centriole and acquisition of the ability to act as a basal body for a primary cilium, and it occurs at the G2/M transition. Maturation is distinct and separable from the actual G1/S centriolar duplication event. Addition of the proteins to the spindle pole is also correlated with modification state. Phosphorylated forms of centrin (J. Salisbury, Mayo Clinic, Rochester, Minnesota) and Spc98p (E. Schiebel) appear to identify the pole-resident populations of these proteins. However, it is not yet known if the phosphorylation of these proteins is required for assembly into the spindle pole and/or is required for some function once at the pole.

On the regulatory side, the *S. cerevisiae* Mps1p protein kinase was reported to be activated and inactivated during late G1 when SPB duplication is occurring, consistent with its role in SPB duplication (M. Winey, University of Colorado, Boulder, Colorado). Two new yeast genes, *UPS1* (M. Rose) and *PCS1* (B. Byers, University of Washington, Seattle, Washington), involved in *S. cerevisiae* SPB duplication were reported. Interestingly, *PCS1* encodes a cap component of the 19S proteasome, implicating proteolysis, required in several cell cycle transitions, as important for SPB duplication (McDonald and Byers, 1997). Lastly, mutations in the *C. elegans* *zyg-1* gene were shown to block centrosome duplication in the embryo (K. O'Connell, University of Wisconsin, Madison, Wisconsin). Studies of the synthesis, modification and assembly of organizing center components are just now being combined with careful tracking of the activities of regulatory molecules. The temporal relationships so revealed may suggest dependency relationships between components and regulators that can be tested directly.

On the cell cycle approach to duplication, two groups reported on experiments in embryonic systems that addressed how the master regulators of the cell cycle are involved in triggering organizing center duplication. Previous work had shown that centrosome duplication in frog and sea urchin eggs continued in the absence of protein synthesis, under conditions in which mitotic cyclins, which are normally degraded and then resynthesized, were at very low levels (Gard et al., 1990; Sluder et al., 1990). More recent work has shown that levels of cyclin E, a G1/S cyclin, are constant in many embryonic cells and thus unaffected by inhibiting protein synthesis. K. Reynolds (Stanford University, Palo Alto, California), and T. Stearns reported that blocking cyclin E/cdk2 activity (cdk2 is the protein kinase with which cyclin E is associated) in these embryos by injection of the p21 cdk inhibitor protein blocked the continued rounds of centrosome duplication, implicating cyclin E/cdk2 as the kinase driving centrosome duplication. Also by manipulating cdk activities, this time in sea urchin eggs, G. Sluder (Worcester Foundation, Shrewsbury, Massachusetts) determined that cells held at a mitotic arrest do not duplicate their centrosomes. When released, but prevented from entering the next round of DNA synthesis, the centrosomes duplicate only once. However, when held at an S-phase arrest, the zygotes will repeatedly duplicate their centrosomes suggesting that not only is S phase permissive for centrosome duplication but "licensing" can happen at this point to allow the multiple rounds of duplication. It appears that a general cell cycle regulator whose activity is required for centrosome duplication has been identified and the timing of that activity is being refined. The next challenge is to define the downstream targets of this regulator, thus bringing together the work on the cell cycle and on the organelle itself.

#### ***The Centrosome Is Dead; Long Live the Centrosome!***

There has been something of a revolution in understanding of the microtubule cytoskeleton in recent years. The centrosome has been knocked from its perch as the unique organizer of microtubules, replaced in part by activities

that can perform some of the same roles. The radial aster is the basic unit of microtubule organization in animal cells and has been assumed to result from the nucleation of microtubules by the centrosome and the continued attachment of those microtubules to the centrosome. Several results call this simple model into question. First, G. Borisy (University of Wisconsin, Madison, Wisconsin) presented evidence that microtubules become detached from the centrosome, moving into the cytoplasm and eventually depolymerizing (Keating et al., 1997). It has long been known that some, possibly many, of the hundreds of microtubules in the typical interphase animal cell do not have their minus ends associated with the centrosome, and this release phenomenon was the first direct evidence for how this situation occurs. It is not known whether the released microtubules have centrosomal material, such as  $\gamma$ -tubulin, associated with their minus ends.

If many microtubules do not have either of their ends associated with the organizing center, then how is an astral arrangement of microtubules maintained? Several lines of evidence suggest that a combination of cellular geometry, dynamic properties of microtubules, and microtubule motors act with the centrosome to maintain the microtubule array. T. Surrey and S. Leibler (Princeton University, Princeton, New Jersey; Nedelec et al., 1997) presented evidence that solutions of pure tubulin and cross-linked microtubule motor proteins are able to form a diverse array of microtubule structures, including simple asters that look remarkably like centrosomally nucleated asters. This work was an extension of previous experiments that had shown that mitotic *Xenopus* egg cytoplasm has the capacity to form centrosome-free asters, dependent on microtubule motor activity and mitotic cell cycle state (Verde et al., 1991). Such asters do however have several centrosomal components at their center (Stearns and Kirschner, 1994). More recently, it has been shown that bipolar spindles can form in the absence of centrosomes in *Xenopus* egg cytoplasm (Heald et al., 1996; Merdes and Cleveland, 1997), and W. Sullivan (University of California, Santa Cruz, California) presented results showing that the same is true in parthenogenetic *Sciara* embryos, although the anastral spindles that formed were unable to remain separate in these syncytial embryos.

Another attribute of the centrosome is indicated by its name; the centrosome is often found at the center of the cell. G. Borisy presented work showing that acentrosomal fragments of fish pigment cells, packed with pigment granules that move on microtubules, are able to generate an astral array of microtubules when pigment granule aggregation is induced (Rodionov and Borisy, 1997). Remarkably, this array is usually located in the center of the cell, suggesting that a radial array of microtubules has a self-centering activity, with or without an associated centrosome. These cell fragments must have a means of nucleating microtubules without a centrosome, and it is possible that the pigment granules themselves are able to nucleate, although it has not yet been determined whether  $\gamma$ -tubulin is associated with the granules.

With all these demonstrations of centrosome-like activities in the absence of centrosomes, one is left wondering what it is that the centrosome does do. There is

little doubt that the centrosome is required for these events to occur efficiently. J. Kilmartin summed this up nicely, stating "Given the choice of having my cells divide with or without centrosomes, I know which one I would choose." The bottom line is likely to be that the centrosome is the driving force in microtubule organization, but that it gets a lot of help in this task from microtubule-associated proteins and microtubule motors. Also, most of the experiments described above were done in systems that have very special properties; this does not diminish the importance of the experiments, but does make it difficult to generalize from the results. An interesting approach to generating cells without functional centrosomes was described by A. Khodjakov (Wadsworth Center, Albany, New York) and C. Rieder. Transient expression a  $\gamma$ -tubulin-GFP (green fluorescent protein) fusion protein allowed them to visualize the centrosome in living cells. Once located, a laser was used to ablate the centrosome (Khodjakov et al., 1997). When analyzed, these cells will provide a look at what happens in cells that are more typical of animal somatic cells.

#### ***The Centrosome in Development and Heredity***

The importance of the male and female genomes at fertilization is obvious, but less attention has been paid to the equally important role of the centrosome in fertilization. For example, much has been written concerning the origin of Dolly the famous sheep's two parental genomes, yet the source of the centrosome in that famous fertilization went without mention (Wilmut et al., 1997). This is a serious oversight because it is well-established that in most animals the sperm brings the first postmeiotic centrosome (or at least the essential initiator of it) to the egg, and that this centrosome is essential to development.

R. Palazzo reported on the behavior of the sperm centrosome during fertilization in clam, using the newly identified component SpiCen300 as a marker for the active centrosome. SpiCen300 is not present on sperm, but is recruited during formation of the sperm centrosome to the sperm's basal bodies during meiosis I. Coincident with this recruitment, the paternal centrosome forms an aster. Remarkably, the paternal centrosome loses both SpiCen300 and microtubule nucleation function at metaphase of meiosis I, whereas the maternal centrosomes in the same cytoplasm remain active. Palazzo suggested that this differential regulation of maternal and paternal centrosomes exists to coordinate the completion of meiosis, which happens well after fertilization in clam, and parental centrosomal inheritance.

The centrosomal events of human fertilization were only recently determined (Simerly et al., 1995), and G. Schatten (Oregon Health Sciences University, Portland, Oregon) discussed further work on humans and other mammals. One of the most surprising results was that the ability of sperm to form a sperm aster was often correlated with how "good" the sperm were at making a successful fertilization. These results have important implications for understanding some types of infertility and identify a potential target for controlling fertility.

Once the embryo is off and dividing, the centrosomes play a role in providing spatial information between the microtubule cytoskeleton and the cell surface. This has been best determined in *Drosophila* where the early

divisions take place in a syncytium, which cellularizes after the nuclei and centrosomes move to the surface and divide several times. T. Kaufman (Indiana University, HHMI, Bloomington, Indiana) talked about centrosomin, the product of the *arr* locus in flies. Centrosomin appears to have two roles, one in the microtubule cytoskeleton as a protein that is localized to the centrosome during mitosis, another as a signal transduction protein, involved in transduction of the wingless signal. Embryos lacking centrosomin fail to cellularize and have aberrant spindles during cleavage. Indeed these embryos seem to lack normal centrosomes altogether and make anastral spindles similar to those described in the parthenogenetic *Sciara* embryos above. Along these lines, W. Sullivan presented evidence that the *Drosophila* Nuf (nuclear fallout) protein is localized to the centrosome during prophase of the early divisions and is required for normal recruitment of actin to the furrows that form during metaphase, suggesting a functional link between the actin cytoskeleton and the centrosome.

#### ***Looking Back, Looking Forward***

A modern cell biologist is struck by the space Wilson devoted to the centrosome in his famous 1925 text, especially considering that much of the work was done by Boveri and colleagues more than 20 years earlier. It is striking both because of the quality of the information—much of what we know about the centrosome during fertilization still derives from these careful studies—and the quantity—most modern cell biology texts offer little to compare. The relative paucity, until recently, of new information could be directly attributed to the traditional difficulties of working with the microtubule organizing center: small amounts of material and an unsure relationship between the animal cell centrosome and the morphologically different SPB found in the advanced genetic model systems. These difficulties have now been largely overcome. Large quantities of centrosomal material can be purified (although this is still not easy), and there are obvious homologies between centrosomes and SPBs that make it possible to glean general information from detailed genetic studies.

Continued study of centrosomes and SPBs will lead to an understanding of how they are produced and how they function, and will also help to determine how these processes can go awry in disease states and other maladies. Infertility can, in some cases, be traced to defects in reconstitution of centrosome function from the sperm basal body (G. Schatten). Both S. Doxsey and J. Salisbury reported that centrosome defects including increased size and number, as well as supernumerary centrioles, and other defects, were common in carcinoma cells from various tumors. These observations suggest that these cells had lost control of normal centrosome duplication and integrity, such that it is easy to imagine these cells displaying genomic instability because of severe defects in centrosome assembly and behavior that negatively impact mitotic spindle assembly and function. Given the importance of genomic instability in the ontogeny of cancer, Bruce Alberts (NAS, Washington D.C.; UCSF, San Francisco, California) encouraged the community to consider these observations as the basis for therapeutic approaches. A heady goal for the next 100 years.

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