Commentary

Anaphase *in vitro*

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Despite their varied appearances, all mitotic spindles have a similar architecture: they are organized as two half spindles, and the microtubules in each half spindle are of the same polarity, with many of the plus ends of the microtubules that grow out from the poles interacting with chromosomes or the microtubules of the other half spindle. During anaphase, the chromosomes move to the spindle poles as the kinetochore-attached microtubules depolymerize (anaphase A), and the spindles elongate due to the presence of pushing forces in the spindle midzone and pulling forces acting on the spindle poles (anaphase B).

Although Mazia and Dan (1) first isolated the mitotic apparatus from sea urchin zygotes over 40 years ago, still left unanswered are many questions regarding the identities, locations, and rearrangements of the structural, mechanochemical, and regulatory molecules involved in chromosome separation. Even with a plethora of genetic mutants in various organisms, to determine the nature of these mutants and the relative roles of the affected gene products during mitosis, it is essential to have functional *in vitro* model systems that can be used to dissect and then reconstitute those elements of the spindle responsible for chromosome movement.

The mitotic spindle has many unique features as an organelle that make it difficult to analyze with biochemical techniques. First, the spindle is a dynamic structure; the assembly and disassembly of microtubules contributes to the force generation mechanism responsible for chromosome separation (2). As an analysis of the history of mitosis *in vitro* shows, media that stabilize spindle structure against the rigors of organelle isolation often inactivate the motile apparatus and render an analysis of the role of microtubule dynamics moot (3). A second problem is that multiple mechanisms of force generation contribute to chromosome separation. Furthermore, redundant motor proteins may participate in any one mechanism of force generation. This has come as a shock to many cell biologists, especially those who tried to explain how chromosomes move by evoking just one mechanochemical system as responsible for all aspects of chromosome movement (reviewed in ref. 4). The current inventory of microtubule motors in the cell is very large, and many of them appear to contribute toward driving the assembly of the spindle apparatus and the subsequent chromosome movement it supports. Thus, spindles are much more complex mechanically than we ever suspected, making a biochemical dissection of spindle function difficult.

In a report in this issue of the *Proceedings*, Murray *et al.* (5) describe anaphase chromosome movements in *Xenopus* egg extracts. Several unique features of these extracts allowed Murray *et al.* to circumvent many of the problems inherent in studying mitosis *in vitro*. Conveniently, spindles self assemble in the frog extracts (6) without the addition of microtubule-stabilization agents. These extracts are undiluted cell sap minus nuclei and storage granules and are much in favor with the cell cycle field because they can readily reproduce key aspects of the cell cycle *in vitro*. Cell extracts are prepared by using low speed centrifugation to gently crush unfertilized frog eggs that are arrested in metaphase of meiosis I. If chromosomes or interphase nuclei are added to the extracts, spindles

form (6). The microtubules in these spindles display properties similar to that observed in intact cells: they are dynamically unstable and the microtubules display a flux due to loss of subunits at the spindle poles as new subunits are added on at the kinetochores (7). Reentry into the cell cycle can be initated by addition of calcium to the extracts. Although the details of how calcium triggers the transition to interphase are not well understood, after calcium addition maturation promoting factor inactivation via cyclin destruction occurs and the connections between sister chromosomes are severed, allowing anaphase chromosome separation to occur (8).

Murray *et al.* (5) measure the kinetics of anaphase chromosome separation and demonstrate that this movement occurs independently of maturation promoting factor inactivation. What is important about these observations is that Murray *et al.* demonstrate that both anaphase A and anaphase B occur in these extracts and are readily accessible to experimental manipulation. Except for the limited chromosome segregation shown to occur after permeabilization of mammalian tissue culture cells (9), previously it has not been possible to support both anaphase A and B *in vitro* in the same preparation.

The long history of spindle isolation procedures developed prior to this study is most readily described in terms of the agents used for spindle stabilization (see ref. 3 for a review of early efforts at spindle isolation). The first method by Mazia and Dan (1) relied on cold ethanol to stabilize the spindle and detergents to extract contaminants. This method was quickly acknowledged to be undesirable because the stabilization was, in essence, irreversible. Ultimately Kane (10) found that acidic pH and any in a series of glycols, such as hexylene glycol, would give a useful stabilization of spindle components. However, spindle isolates prepared by these techniques have been of limited usefulness since they have neither the lability properties of *in vivo* spindles nor do they move chromosomes. Their chief value has been to demonstrate that the spindle is a discrete organelle with many fibrous elements—i.e., microtubules.

A renewed interest in spindle isolation occurred after the discovery of microtubule polymerization conditions. Sakai *et al.* (11) isolated spindles from sea urchin eggs that were cold and Ca liable, and when incubated in tubulin, spindle birefringence (a measure of the number of microtubules in the spindle) increased. Subsequently, Palazzo *et al.* (12) were able to use a similar isolated sea urchin preparation to support anaphase spindle elongation by the addition of tubulin and either GTP or ATP. However, neither preparation supported a chromosome-to-pole movement equivalent to that which occurs normally during anaphase A.

Isolated diatom spindles have been important model systems for analyzing the mechanism of anaphase spindle elongation. The diatom central spindle is uniquely suited for the study of the mechanism of anaphase B because the fibrous systems responsible for anaphase A and B are spatially separated and the central spindle, the structure responsible for spindle elongation, is a paracrystalline array of microtubules with a prominent, well defined zone of microtubule overlap (13). This overlap zone is visible even by light microscopy and it de-

creases in extent as the spindle elongates. We have developed simple procedures for isolating central spindles from dividing cells of the large centric diatom *Stephanopyxis turris* and the small pennate diaton *Cylindrotheca fusiformis* (14, 15). With the addition of ATP and in the absence of tubulin, Cande and McDonald (14) were able to reactivate anaphase B movement in isolated spindles that lacked cytoplasmic connections, showing that mechanical interactions in the zone of microtubule overlap can drive spindle elongation even in the absence of tubulin polymerization. Both pharmacological and immunological evidence suggested that a kinesin-related protein (krp) is responsible for this event in the central spindle (16). Using a variety of strategies, krps have also been implicated as playing a role in anaphase B in a variety of organisms including budding yeasts (17) and mammalian cells (18). Subsequently, we have identified an 85-kDa protein diatom spindle kinesin 1 (DSK1) that is involved in mitotic spindle elongation in the diatom *C. fusiformis* (15). DSK1 was first isolated using a peptide antibody raised against a conserved region in the motor domain of the kinesin superfamily that stained the diatom spindle (16). Polyclonal antisera raised against a non-conserved region of DSK1 stains the diatom central spindle with a bias toward the midzone (Fig. 1), and immunoblots show that the protein is greatly enriched in isolated spindle prepa-
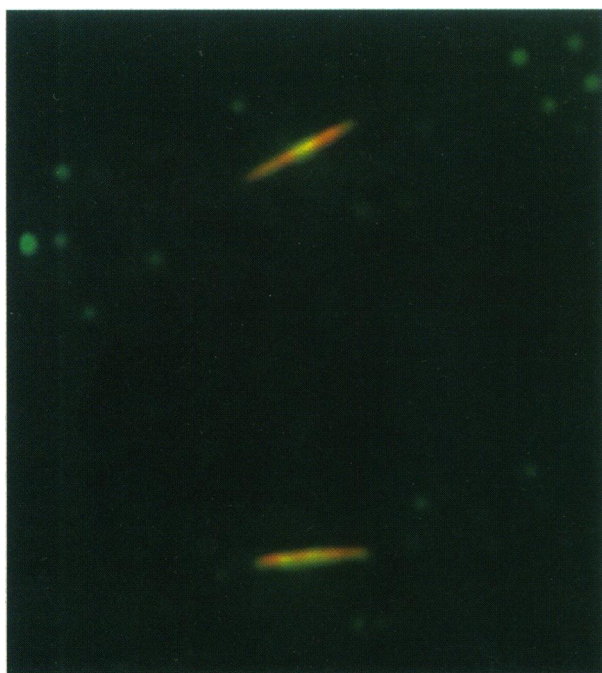

FIG. 1. Double immunofluorescent staining of permeabilized diatom cells before spindle reactivation showing the relative distribution of DSK1 (green) and tubulin (red). DSK1 is concentrated in the zone of microtubule overlap, which appears bright yellow due to the superimposition of the two labels. The image is an overlay from a single optical section after three-dimensional deconvolution. The spindles are approximately 3 μm long.

rations. Furthermore, DSK1 antibody blocks spindle elongation *in vitro* (15).

To date, frog extracts have been most useful for studying events early in the mitotic progression, including nuclear envelope breakdown, chromosome condensation, and spindle formation. However, Murray *et al.* (5) show now that these extracts can be used to analyze late stage events such as anaphase chromosome segregration. Although frog extracts are almost as complex as the egg itself, recent studies from the Mitchison and Karsenti laboratories (19, 20) demonstrate that it is possible to use a combination of molecular strategies to dissect spindle function in frog extracts. By using immunodepletion and confirming with add-back experiments, Walczak *et al.* (19) showed that the krp, XKCM1, regulates microtubule dynamics during spindle assembly. Boleti *et al.* (20) use a different strategy to show that a novel krp, Xklp2, is required for spindle pole separation during spindle assembly. In their studies recombinant Xklp2 mutants are used to demonstrate that the C-terminal tail of the molecule is responsible for targeting the krp to the spindle. Xklp2 molecules lacking the motor domain act as a dominant negative mutation, blocking centrosome separation during spindle assembly and disrupting preassembled metaphase spindles. Thus both strategies circumvent the need to isolate spindles from the frog extract or to laboriously fractionate the extract by conventional biochemical techniques. Considering the work of Murray *et al.* (5) described in this issue of the *Proceedings*, similar techniques could now be used to dissect the mechanochemical basis of anaphase A and B and its regulation.

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