Mini-Review

Functional Redundancy in Mitotic Force Generation

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THE pushing and pulling forces that characterize the process of mitosis have fascinated and frustrated biologists for over a century. While considerable progress has been made in understanding how these forces are used in mitosis, the nature of the molecules that generate the forces has remained obscure. Several recent papers (Hoyt et al., 1992; Roof et al., 1992; Saunders and Hoyt, 1992), including one in this issue of *The Journal of Cell Biology* (O'Connell et al., 1993), have demonstrated that members of the kinesin superfamily of microtubule motors are likely to generate some of these forces. Intriguingly, the functions of some of these molecules are nonessential. Coupled with recent demonstrations that dynein (Pfarr et al., 1990; Steuer et al., 1990) and microtubule depolymerization (Koshland et al., 1988; Coue et al., 1991) could also produce spindle forces, these observations raise the possibility that spindle force generation is a highly redundant process.

Three of the most obvious mitotic movements and forces occur during prophase, metaphase, and anaphase (reviewed in McIntosh and Pfarr, 1991). First, during prophase, the newly duplicated spindle poles or centrosomes separate to form the bipolar mitotic spindle. Second, during anaphase, chromosomes move to the spindle poles as a result of pulling forces generated between the chromosomes and the spindle poles. At metaphase, when chromosomes are aligned at the middle of the spindle, a similar force may also cause poles to be pulled towards one another (see Fig. 1). Third, also during anaphase, spindle poles separate from one another as the spindle elongates. This movement appears to be largely driven by forces intrinsic to the spindle that push the spindle poles apart. This force may also counterbalance the force generated between chromosomes and poles at metaphase, and thus keep the spindle from collapsing. All of these forces may, at least in part, be generated by members of the kinesin superfamily of proteins.

The kinesin superfamily was defined by its founding member, the kinesin heavy chain, which is the principal component of the microtubule-dependent motor protein kinesin (Vale et al., 1985). This protein was first found in squid axoplasm but was subsequently found to be virtually ubiquitous, which led to the view that kinesin itself might produce the force driving some of the various mitotic movements (reviewed in Sawin and Scholey, 1991). While this view could still have some validity, the bulk of recent evidence suggests that kinesin itself may not play a role in mitosis. Instead, a group of proteins distantly related to kinesin heavy chain, called kinesin-like proteins or kinesin-related proteins, appear to play key roles in mitosis. These proteins are all related to kinesin heavy chain by virtue of having substantial sequence similarity only to the motor region of kinesin heavy chain. Interestingly, these proteins each have unique "tail" domains attached to the putative motor domain. These tail domains may confer functional diversity on this large superfamily of proteins (reviewed in Goldstein, 1991).

In *Saccharomyces cerevisiae* three genes required for normal mitosis have been found to encode members of the kinesin superfamily. The first of these, *KAR3*, was identified by mutations that interfere with the process of nuclear movement during zygote formation in this organism (Polaina and Conde, 1982; Meluh and Rose, 1990). However, in addition to a role in this process, *KAR3* was also inferred to have a nonessential (redundant?) role in mitosis, based on its slow growth, production of nonviable cells, and accumulation of large budded cells with short mitotic spindles. The other two genes are called *CIN8* and *KIP1*. *CIN8* was first identified by mutations that cause elevated rates of chromosome loss (Hoyt et al., 1990, 1992); *CIN8* was also independently found because it caused synthetic lethality in combination with *KIP1* deletions, which have no phenotype of their own (Roof et al., 1992). *KIP1* was found in a polymerase chain reaction (PCR)-based search for genes encoding members of the kinesin superfamily (Roof et al., 1992). *KIP1* was also found as a gene that could rescue the defect in *CIN8* mutants when overexpressed (Hoyt et al., 1992). As just mentioned, *KIP1* deletions have no phenotype when present as single mutants, while *CIN8* deletions have a mild chromosome loss phenotype at 26°C, and a severe chromosome loss phenotype and viability defect at 37°C.

Had *KAR3*, *CIN8*, and *KIP1* only been studied in isolation, one would have concluded that each of these genes encode motor proteins that play an unimportant role in mitosis, since deletions of each gene are viable (at least at 26°C). Producing double and triple mutants among these, however, demolished such a view. For example, the consequence of removing both *CIN8* and *KIP1* function before spindle assembly is to block the separation of spindle poles during mitosis such that a bipolar spindle is not formed (Hoyt et al., 1992; Roof et al., 1992). Removing *CIN8* and *KIP1* function after spindle assembly, but before anaphase starts, leads to spindle collapse (Saunders and Hoyt, 1992). A surprise comes when *KAR3 CIN8 KIP1* triple mutants are produced. These triple mutants are healthier than *CIN8 KIP1* double mutants, at least in part because the removal of *KAR3* function partially rescues the spindle collapse phenotype seen in *CIN8 KIP1* double mutants (Saunders and Hoyt, 1992). Thus, *KAR3* function somehow compensates for the loss of

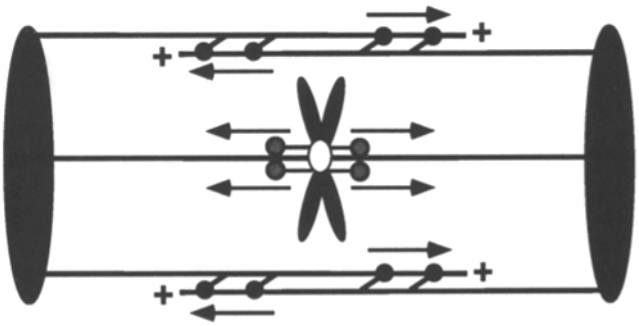

Figure 1. Schematic view of metaphase of mitosis to illustrate directions of various forces and movements. The solid black ovals represent spindle poles. The black lines represent microtubules with plus ends distal to the spindle pole. A chromosome (not drawn to scale) is shown at the equator of the spindle with connections to microtubules from both poles; the open oval represents the kinetochore. The solid black circles with stems represent microtubule motors (CIN8 or KIP1?) bound to one microtubule by a static linkage (*stem*) and with the motor (*solid black circle*) bound to another microtubule. When the motor elements attempt to translocate toward the plus end of the microtubule (indicated by *adjacent arrow*), a force causing spindle poles to separate is generated. The shaded circles with stems represent microtubule motors (KAR3 or dynein?) bound to a kinetochore with a static linkage (*stem*) and with the motor (*shaded circle*) bound to a microtubule connecting the chromosome to the pole. When the motor attempts to translocate toward the pole, this will generate a force pulling the chromosome toward the pole, or the pole toward the chromosome; the latter might be counterbalanced by the pole separation force to ensure that the spindle poles do not collapse together. At anaphase when the sister chromatids split, chromosome to pole movement will ensue. Microtubule depolymerization at the kinetochore could generate a similar force.

CIN8 and *KIP1* function. Taken together, these observations suggest that *CIN8*, *KIP1*, and *KAR3* all encode motors that are active participants in mitotic spindle function. Consistent with this notion is the observation that both CIN8 and KIP1 proteins are located in the mitotic spindle; the location of the *KAR3* protein remains obscure.

While a variety of models could explain these observations, a very provocative model was proposed by Saunders and Hoyt (1992). In this model, the CIN8 and KIP1 proteins are suggested to produce forces that push spindle poles apart, perhaps by cross-bridging spindle microtubules and generating forces directed away from the spindle pole itself (see Fig. 1). In this view KAR3 is thought to generate forces pulling the poles together, thus counteracting the CIN8 and KIP1 generated forces. Where KAR3 generates its forces is obscure, but one intriguing possibility is that it generates forces pulling the chromosomes and the poles together. This possibility in turn might suggest that KAR3 provides some of the force needed for chromosome–pole movement; if true, this force must be redundant since *KAR3* deletions are viable.

These types of phenomena are not limited to *S. cerevisiae*. Mutations in the *bimC* gene of *Aspergillus nidulans* also cause defects in spindle pole segregation during mitosis (Enos and Morris, 1990). Consistent with the functional similarity, the motor domain of the *bimC* product is more similar in sequence to the CIN8 and KIP1 motor domains than to those of other kinesin superfamily members. In agreement with the story in *S. cerevisiae*, *bimC* mutants are suppressed by deletions of the *A. nidulans klpA* gene, which may encode a KAR3 homologue (O'Connell et al., 1993). The KLPA protein has analogous predicted structural features to the KAR3 protein, although they have no detectable sequence similarity outside of their presumptive motor domains. Consistent with the view that *klpA* and *KAR3* are homologues, *klpA* can partially rescue defects in *KAR3* mutants. Somewhat surprisingly, *klpA* deletions are fully viable on their own and show no detectable phenotype, although *klpA* overexpression leads to mitotic arrest. Taken together, the data suggest that the *klpA/bimC* interaction may have the same basis as the *KAR3/CIN8/KIP1* interactions. A similar situation could exist with the *Schizosaccharomyces pombe cut7* gene (Hagan and Yanagida, 1990), although no *KAR3/klpA* homologue has yet been found.

While these data might suggest that motors of the kinesin superfamily are all that are used to produce mitotic forces, recent work suggests that other force producers are also used. The microtubule motor protein dynein, which is strikingly distinct from kinesin in sequence and biochemical properties (Gibbons et al., 1991; Ogawa, 1991), has been found in mitotic spindles and kinetochores (Pfarr et al., 1990; Steuer et al., 1990), where it has been suggested to produce mitotic forces. Microtubule polymerization and depolymerization have also been suggested to produce mitotic forces for spindle pole separation and chromosome to pole movement, respectively (for review see Inoue, 1981); the latter suggestion has recently been shown to be physically plausible (Koshland et al., 1987; Coue et al., 1990).

What are we to make of all this? While it is possible that the simple interpretations of one, or all, of these observations is incorrect, or that different organisms and spindles rely on different force generating systems, it may be that spindles truly use multiple redundant force generating mechanisms. The viability of *klpA* and *KAR3* deletions, their suppression of *bimC* and *CIN8/KIP1* defects, and the obvious redundancy of *CIN8* and *KIP1* all suggest that these genes encode force generating molecules with overlapping and counterbalancing functions. If, in fact, KAR3 produces forces pulling chromosomes to the poles as suggested (Saunders and Hoyt, 1992), then these forces could also be redundant with the forces generated by dynein, or by microtubule depolymerization.

Why might there be functional redundancy in the generation of mitotic spindle force? One possibility is that these forces are used for distinct, but overlapping and coordinated movements that are required to generate the extraordinarily high fidelity of chromosome segregation ($<10^{-5}$ mistakes per cell division; Hartwell and Smith, 1985). An obvious example is anaphase A, which involves chromosomes moving toward the poles, and anaphase B, which involves poles moving apart; both contribute to a net segregation of chromosomes from each other. A second possibility is that these apparently redundant forces are only nonessential when an organism is living in the laboratory, but they are required in the outside world when conditions are not so favorable. A final possibility, related to the previous one, is that these functions are not required now but they were required during evolution, perhaps in different environmental conditions. In this context it is worth remembering that it is possible for small increases in selective fitness, on the order of 1%, to

lead to evolutionary fixation of the alleles causing the elevation in fitness (Crow and Kimura, 1970).

However one looks at the reasons for functional redundancy of mitotic spindle forces, the simple view of mitotic spindle force generation, where each movement is powered by a single motor, is clearly no longer adequate. Instead, we may now have to accept that cells have evolved so that coordination and redundancy of multiple motor molecules in mitosis are the rule.

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