Comment

Microtubules in *orbit*

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The faithful segregation of DNA during mitosis is critical to the life of a cell. A complex molecular machine called the mitotic spindle mediates this task. The spindle is made up of microtubule-binding proteins and microtubules on which the chromosomes are segregated. A complete understanding of the formation of the bipolar spindle and the mechanism of chromosome movement on the spindle remains elusive. Many key players in this process have been identified, and we are beginning to understand the various classes of proteins and their functions. However, not surprisingly, new players with potentially novel modes of action continue to be discovered. In this issue, Inoue and colleagues describe a novel microtubule-associated protein encoded by the *Drosophila* gene *orbit* that influences the structure of the spindle and binds microtubules in a GTP-dependent manner (Inoue et al., 2000).

Microtubules are dynamic polymers made up of α,β-tubulin heterodimers. Microtubules exhibit a phenomenon called dynamic instability, wherein they coexist in states of growth and shrinkage (reviewed in Desai and Mitchison, 1997). Many different proteins have been discovered that bind to microtubules (reviewed in Kreis and Vale, 1999), including microtubule stabilizers and destabilizers, microtubule severing proteins, and motor proteins. It is the balanced interplay between these microtubule-binding proteins that enables the dramatic, coordinated alterations of microtubules necessary for spindle formation and chromosome movements during mitosis. One specialized group of binding proteins called microtubule-associated proteins (MAPs)[1] enhances the stability of microtubules (reviewed in Cassimeris, 1999). Although MAPs were initially characterized in neurons, several non-neuronal MAPs important in regulating cellular microtubule dynamics have been identified. These MAPs appear to be highly regulated during interphase and mitosis; upon inactivation a MAP is no longer able to bind to microtubules, and its stabilizing activity is reduced.

Because of the importance of microtubule stability in the spindle, it is not surprising that multiple MAPs exist that are essential during mitosis. Inoue and colleagues report the discovery of the *Drosophila* gene *orbit*, which encodes a novel MAP (Inoue et al., 2000). The *orbit* mutation was isolated in a screen for maternal effect lethal genes. Characterization of spindle and DNA morphology in the syncytial embryo revealed free centrosomes, multipolar spindles, and curved or bent spindles (Fig. 1 a), phenotypes that are consistent with defects in chromosome segregation. Somatic cell defects were also observed in the larval central nervous system (CNS), including circular mitotic figures (CMFs), polyploidy and hypercondensed chromosomes, as well as a small number of monopolar spindles with chromosomes in an anaphase-like configuration (Fig. 1 b). In addition, they observed that *orbit* mutants were delayed in progression through the cell cycle, which is consistent with the hypercondensed chromosomes that were observed.

The 165-kD product of the *orbit* gene was isolated and contains a highly basic central region, characteristic for microtubule-binding proteins (Fig. 2). This region contains two consensus sites for phosphorylation by $p34^{cdc2}$ that may be important for regulating the association of Orbit with microtubules. Sequence analysis of the putative microtubule-binding domain revealed that it is similar to that of MAP4, a nonneuronal mammalian MAP important in stabilizing interphase microtubules, and to Stu1, a MAP found in budding yeast. Consistent with these findings, Orbit was found to cosediment with microtubules in embryo extracts. In addition, immunolocalization of Orbit showed that it colocalized with spindle microtubules suggesting a role in stabilizing microtubules during mitosis.

Perhaps the most interesting finding reported by Inoue and colleagues is that Orbit appears to bind microtubules in a GTP-dependent manner. Sequence analysis indicates the presence of two putative GTP-binding motifs within the highly basic central region of Orbit. These motifs closely resemble those found in β-tubulin and other members of the GTPase superfamily (Downing and Nogales, 1998; Nogales et al., 1998). Orbit was shown to bind to microtubules in a GTP-dependent manner in blot overlay assays. Consistent with this observation, microtubule pelleting assays indicate that Orbit must bind GTP to cosediment with microtubules. MAPs are known to bind and release microtubules; however, this is usually dependent on salt concentration and is often regulated by phosphorylation. If Orbit can bind and release from microtubules in a nucleotide-sensitive manner, this would be a unique MAP function, suggesting the possibility of a nucleotide-regulated association with microtubules. Other microtubule-binding proteins that also bind nucleotide include a vast array of motor proteins important in spindle organization (Goldstein and Philp, 1999). The recent identification

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[1]*Abbreviations used in this paper: asp,* abnormal spindle; CMFs, circular mitotic figures; CNS, central nervous system; MAPs, microtubule-associated proteins.

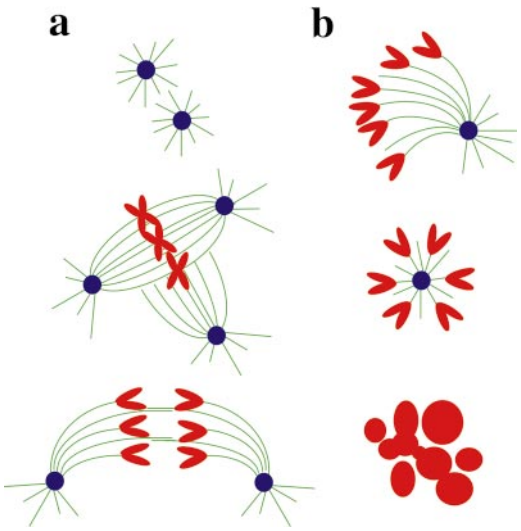

Figure 1. Mitotic defects in *orbit* mutants. Microtubules are illustrated in green, centrosomes in blue, and DNA in red. (a) In syncytial embryos, defects include (top to bottom) free centrosomes, multipolar spindles, and curved spindles that appeared to be in anaphase. (b) In somatic cells of the larval central nervous system, defects include (top to bottom) monopolar spindles that were anaphase-like, circular mitotic figures, and polyploid nuclei.

of kinesin-related proteins that destabilize microtubules or that act as signaling molecules suggest that motor proteins can have more diverse functions other than microtubule translocation (reviewed in Goldstein and Philp, 1999). Perhaps Orbit is the first example of a more dynamic MAP that functions in the spindle. If Orbit uses its nucleotide-binding domain to regulate its association with microtubules, then it will be interesting to compare its activity to conventional motor proteins.

The preliminary model put forth by Inoue and colleagues suggests a primary role for Orbit in regulating the function of spindle microtubules. The high percentage of polyploid cells and the defects in spindle structure are consistent with the idea that Orbit provides stability to spindle microtubules that is lost in the mutants. The hypercondensed chromosomes in larval CNS cells suggest that *orbit* mutants remain blocked in metaphase, which is not surprising if spindle stability is compromised.

It may also be possible that Orbit has a less direct role in centrosome separation during mitosis. Several *orbit* mutant phenotypes resemble those found in other *Drosophila* mutants that block spindle pole separation, such as *aurora* and *merry-go-round* (Gonzalez et al., 1988; Glover et al., 1995). In addition, the bent spindles and monopolar spindles in *orbit* mutants are reminiscent of those seen in *abnormal spindle* (*asp*) mutants (Gonzalez et al., 1990); the Asp protein has recently been shown to be important for proper spindle pole structure (do Carmo Avides and Glover, 1999). Unlike other spindle pole mutants, however, *orbit* mutants can occasionally form a normal bipolar spindle, suggesting that a defect in centrosome separation is not the primary defect in these mutants. One can easily envision how a decrease in microtubule stability could ultimately lead to spindle collapse, resulting in the appearance of a monopolar spindle. It will be interesting to employ time-lapse microscopy of early embryos as they proceed through the early divisions to determine how the spindles in *orbit* mutants are assembled and disassembled.

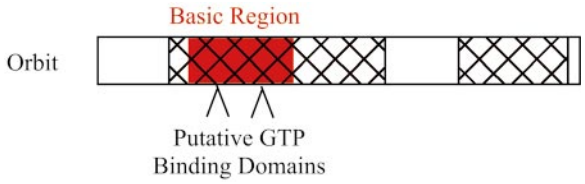

Figure 2. Schematic of Orbit domains. Hatched areas indicate regions of sequence with putative homologues in *C. elegans* and humans. The highly basic region is illustrated in red. This region includes two putative microtubule-binding domains, as well as two putative GTP binding domains.

Future experiments aimed at elucidating the mechanism of action of this interesting MAP are numerous. A biochemical analysis of Orbit regulation of microtubule dynamics and an analysis of its GTPase activity will be essential in providing the groundwork for understanding how it functions in the spindle. In addition, generation of transgenes that express constitutively active forms of Orbit may provide insight into how nucleotide hydrolysis functions to regulate spindle dynamics in cells. Finally, the identification of homologues in *C. elegans* (Inoue et al., 2000) will allow a comparison of phenotypes by using RNA-mediated interference to knockout the *C. elegans* protein. The identification of the human homologue (Inoue et al., 2000) will enable the use of cell lines to study the regulation of microtubule dynamics in cells using high-resolution imaging.

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