

# A perfect funeral with no corpse

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**“Indeed, the role in mitosis of the chromosome arms, which carry most of the genetic material, may be compared with that of a corpse at a funeral: they provide the reason for the proceedings but do not take an active part in them.” (Mazia, 1961)**

The purpose of M phase (using a term that incorporates both mitosis and meiosis) is to segregate the genome in an orderly way, but, surprisingly, there is a great deal of controversy over the role played by the chromosomes in events such as spindle assembly and cytokinesis. This controversy may be further stimulated by a paper, in this issue, describing two *Drosophila* mutants in which cells lacking chromosomes can assemble spindles and undergo cytokinesis during meiosis II (Bucciarelli et al., 2003).

In somatic animal cells, dynamically unstable microtubules nucleated at centrosomes sample the cytoplasm in cycles of assembly and disassembly, probing for sites where they will be captured and stabilized. Microtubule capture by sister kinetochores then establishes the bipolar mitotic spindle (Kirschner and Mitchison, 1986). Higher plants and female meiotic spindles of some animals lack centrosomes and use an alternative strategy to assemble bipolar spindles (McKim and Hawley, 1995). In *Xenopus* eggs and extracts, microtubules preferentially assemble in the vicinity of chromosomes (Karsenti et al., 1984), and are then organized into a bipolar spindle by various motor proteins (Wittmann et al., 2001). The chromosome proximity effect can be explained by the presence of a Ran-GTP gradient that causes importins  $\alpha$  and  $\beta$  to release factors critical for spindle assembly (Hetzler et al., 2002). Because the Ran-GEF, RCC1, is a chromosome-associated protein, the Ran gradient is generated near chromosomes.

The Ran gradient may still be essential for spindle assembly even in the presence of centrosomes. However, evidence

against this model has come from two studies in which bipolar microtubule arrays were induced to form in the absence of chromosomes (Brunet et al., 1998; Faruki et al., 2002). In both cases, the spindles were not fully functional, and could not undergo a normal anaphase-like elongation.

Another classic problem of mitosis is the mechanism of positioning and assembly of the cleavage furrow. The classic studies of Rappaport showed clearly that in early embryos, the furrow was positioned by a cleavage stimulus that acted on the cell cortex midway between asters, even in the absence of chromosomes or a central spindle (Rappaport, 1996). Subsequent studies in somatic cells and spermatocytes led to a slightly different conclusion—that the (still unidentified) cleavage stimulus apparently emanated from the central spindle (Cao and Wang, 1996; Bonaccorsi et al., 1998).

Identification of the chromosomal passenger proteins appeared to implicate the chromosomes in the assembly and function of the central spindle and cleavage furrow (for review see Adams et al., 2001). INCENP, the prototypic passenger protein, concentrates in the presumptive cleavage furrow even before myosin II (Eckley et al., 1997). Certain INCENP mutants could block the completion of cytokinesis, as do inhibitors of the Aurora-B kinase (Ditchfield et al., 2003; Silke Hauf and Jan-Michael Peters, personal communication), for which INCENP is a targeting subunit and activator (Adams et al., 2001).

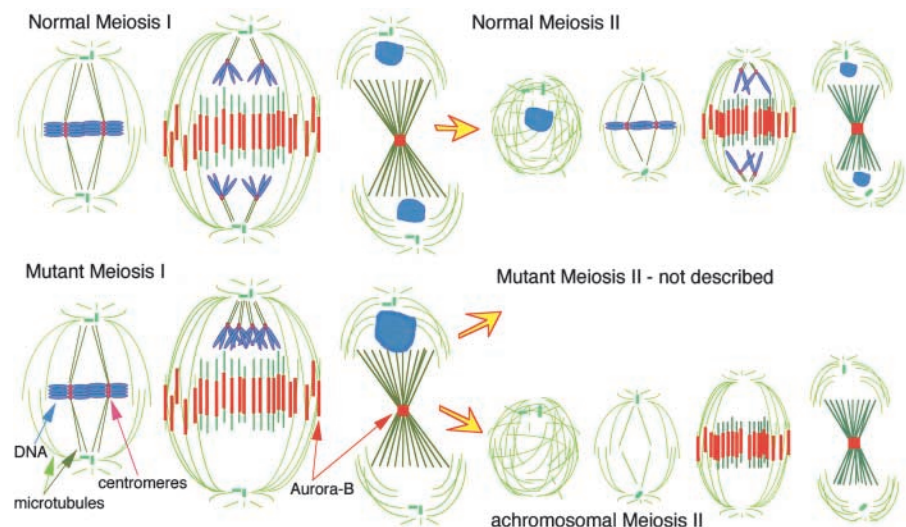
Evidence against a role for chromosomes in cytokinesis came from observations that enucleated sea urchin eggs could duplicate their centrosomes and undergo cycles of mitosis and cleavage (Sluder et al., 1986), and that spermatocytes whose chromosomes had been removed in prometaphase could enter anaphase and undergo cytokinesis (Zhang and Nicklas, 1996). Certain caveats applied, however. Eggs have large stockpiles of many components needed to make chromosomes and spindles, and fragments of the kinetochores could have broken off when the chromosomes were removed surgically. In both cases these factors could have influenced the outcome of the experiment.

The paper by Bucciarelli et al. (2003) has exploited a fortuitous observation to address the role of the chromosomes in spindle formation and cytokinesis. During a screen for *Drosophila* mutants affecting male meiosis, two mutants, *fusolo* and *solofuso*, were obtained. In both, all of the chromosomes partition to one daughter cell ~50% of the time in meiosis I. Remarkably, the achromosomal daughter can enter meiosis II, assemble an apparently normal spindle,

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Figure 1. Diagrams showing the normal events of meiosis I & II, and the unusual events of meiosis II that occur in *Drosophila fusolo* and *solofuso* mutants. Chromosomes (blue); microtubules (green); Aurora B (red). This kinase is initially associated with centromeres but binds to the central spindle after anaphase.



initiate anaphase, organize a central spindle, and undergo cytokinesis—all in the absence of chromosomes! (See Fig. 1.)

This observation appears to argue in compelling terms that (1) a chromosomally-generated Ran-GTP gradient cannot be essential for the assembly of a bipolar spindle in these cells; (2) kinetochores or chromosomes need not be present to form an integrated spindle that can organize a central spindle and signal for formation of the cleavage furrow; (3) localized cleavage of the Scc1/Mcd1/Rad21 cohesin subunit, or any other chromosomal protein for that matter, cannot be essential for the initiation of anaphase; and (4) chromosomes need not be present for the initiation or progression of cytokinesis.

Does this mean that chromosomal proteins such as the chromosomal passengers are unnecessary for late mitotic events? Here, the situation is more complex. Despite the absence of chromosomes, Aurora-B was detected in the spindle midzone and contractile ring of the achromosomal cell. There is a cytoplasmic pool of kinase, and apparently, this can localize in the absence of chromosomes. It had previously been shown that chromosomal passenger proteins can target to ectopic furrows far from the chromosomes in heterokaryons (Savoian et al., 1999).

The generality of these conclusions for other cell types remains to be determined. For example, the dense mitochondria that coat the spindle in *Drosophila* spermatocytes may in some mysterious way substitute for the presence of chromosomes to organize the microtubules. Furthermore, *Drosophila* spermatocytes may “cheat” in certain aspects of their signaling, since cytokinesis at the end of meiosis I is incomplete, and the two meiosis II spermatocytes actually remain joined by ring canals. Nonetheless, the results of this study clearly show that the elegant ritual of the M phase funeral can go on in remarkably normal style, even without the corpse.

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