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 α and β to release factors critical for spindle assembly (Hetzer 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 spermatoocytes 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 acentrosomal daughter can enter meiosis II, assemble an apparently normal spindle,
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.

References

Adams, R.R., M. Carnem, and W.C. Earnshaw. 2001. Chromosomal passengers and the (Aurora) ABCs of mitosis. Trends Cell Biol. 11:49–54.

Bonaccorsi, S., M.G. Giangiacomi, and M. Gatti. 1998. Spindle self-organization and cytokinesis during male meiosis in asters mutants of Drosophila melanogaster. J. Cell Biol. 142:751–761.

Brunet, S., Z. Polanski, M.H. Veilhac, J.Z. Kubiak, and B. Marzo. 1998. Bipolar spindle formation without chromatin. Curr. Biol. 8:1231–1234.

Bucciarelli, E., M.G. Giangiacomi, S. Bonaccorsi, and M. Gatti. 2003. Spindle assembly and cytokinesis in the absence of chromosomes during Drosophila male meiosis. J. Cell Biol. 160:993–999.

Cao, L.-g., and Y.-l. Wang. 1996. Signals from the spindle midzone are required for the stimulation of cytokinesis in cultured epithelial cells. Mol. Biol. Cell 7:225–232.

Ditchfield, C., V.L. Johnson, A. Tague, R. Elston, C. Haworth, T. Johnson, A. Mortlock, N. Keen, and S.S. Taylor. 2003. Aurora B couples chromosome alignment with anaphase by targeting bubR1, Mad2 and CENP-E to kinetochores. J. Cell Biol. In press.

Eckley, D.M., A.M. Ainsztein, A.M. Mackay, I.G. Goldberg, and W.C. Earnshaw. 1997. Chromosomal proteins and cytokinesis: patterns of cleavage furrow formation and inner centromere position in mitotic heterokaryons and mid-anaphase cells. J. Cell Biol. 136:1169–1183.

Faruki, S., R.W. Cole, and C.L. Rieder. 2002. Separating centrosomes interact in the absence of associated chromosomes during mitosis in cultured vertebrate cells. Cell Motil. Cytoskeleton. 52:107–121.

Hetzer, M., O.J. Gruss, and I.W. Maitaj. 2002. The Ran GTPase as a marker of chromosome position in spindle formation and nuclear envelope assembly. Nat. Cell Biol. 4:E177–E184.

Karsenti, E., J. Newport, R. Hubble, and M. Kirschner. 1984. Interconversion of metaphase and interphase microtubule bundles, as studied by the injection of centrosomes and nuclei into Xenopus eggs. J. Cell Biol. 98:1730–1745.

Kirschner, M., and T. Mitchison. 1986. Beyond self assembly: from microtubules to morphogenesis. Cell. 45:329–542.

Mazia, D. 1961. Mitosis and the physiology of cell division. In The Cell. Biochemistry, Physiology, Morphology, J. Brachet and A.E. Mirsky, editors. Academic Press, New York. 77–412.

McKinnon, K.S., and R.S. Hawley. 1995. Chromosomal control of mitotic cell division. Science. 270:1595–1601.

Rappaport, R. 1996. Cytokinesis in Animal Cells. Developmental and Cell Biology Series. Cambridge: Cambridge University Press.

Savoian, M., W.C. Earnshaw, A. Khodjakov, and C.L. Rieder. 1999. Cleavage furrows formed between centrosomes in fused PtK1 cells that lack an intervening spindle and chromosomes contain microtubule bundles, INCENP and CHOP but not CENP-E. Mol. Biol. Cell. 10:297–311.

Sluder, G., F.J. Miller, and C.L. Rieder. 1986. The reproduction of centrosomes: Nuclear versus cytoplasmic controls. J. Cell Biol. 103:1873–1881.

Wittmann, T., A. Hyman, and A. Desai. 2001. The spindle: a dynamic assembly of microtubules and motors. Nat. Cell Biol. 3:E28–E34.

Zhang, D., and R.B. Nicklas. 1996. “Anaphase” and cytokinesis in the absence of chromosomes. Nature. 382:466–468.