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Nondisjunction, aneuploidy and tetraploidy

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One simple, widely accepted mechanism for generating an aberrant chromosome number, or aneuploidy, is through nondisjunction — a chromosome distribution error that occurs during mitosis when both copies of a duplicated chromosome are deposited into one daughter cell and none into the other. Shi and King[1] challenge this view, concluding that nondisjunction does not yield aneuploid cells directly, but instead gives rise to tetraploid cells that may subsequently become aneuploid through further division. Here we show that the direct result of chromosome nondisjunction is gain or loss of a single chromosome, which results in near-diploid aneuploidy, not tetraploidy. We suggest that chromatin trapped in the cytokinetic cleavage furrow is the more likely reason for furrow regression and tetraploidization.

Shi and King use fluorescent *in situ* hybridization to establish a correlation between spontaneous missegregation of chromosomes and binucleation in human cell lines[1]. Notably, they find the same correlation in cells with an intact mitotic checkpoint as in cells in which the checkpoint has been inactivated as a result of depletion of the essential checkpoint protein Mad2. We tested whether nondisjunction *per se* affects completion of mammalian cytokinesis by using recombinase-mediated gene excision to deplete the mitosis-specific motor protein CENP-E from primary fibroblasts. Although mitotic checkpoint signalling is weakened at individual CENP-E-depleted kinetochores, mitosis proceeds as normal in most of these cells, with all duplicated chromatid pairs achieving alignment and then segregation in anaphase. In 25% of divisions, however, one or two duplicated chromosomes fail to make bipolar attachments. These cells sustain a mitotic checkpoint-mediated delay for about an hour, but then anaphase ensues and nondisjunction occurs by retention of both copies of individual, duplicated chromosomes at or near one of the spindle poles[2] (Fig. 1a).

If nondisjunction were to give rise to binucleation and if binucleate cells were to survive as well as mononucleate cells for one cell cycle, as demonstrated by Shi and King[1], then the steady-state proportion of binucleates in the population of CENP-E-depleted cells should triple. Instead, examination of over 500 cells of each genotype revealed no difference in the proportion of binucleation in CENP-E-depleted cells and in wild-type cells (Fig. 1b), which have a nondisjunction rate below 0.5%.

Examination of other cell types from mice supports our conclusion that nondisjunction causes near-diploid aneuploidy. Chromosome counting revealed aneuploidy in 28% of mitotic lymphocytes from mice with reduced levels of CENP-E (compared with 10% in normal mice). This situation is similar in mice with reduced amounts of the mitotic checkpoint protein Bub3, in which 9% of splenocytes are aneuploid compared with fewer than 1% in normal mice[3]. In both cases, almost all instances of aneuploidy reflect the loss or gain of only one or two chromosomes, exactly as would be expected to result directly from simple nondisjunction.

Shi and King also propose that nondisjunction-induced failure of cytokinesis, coupled with tetraploidization and a subsequent aberrant mitosis, underlies the aneuploidy frequently found in human cancer. However, nondisjunction occurs at high frequency in mosaic variegated aneuploidy, a condition characterized by childhood cancers and thought to be caused by mutations in the mitotic checkpoint kinase BubR1 (refs 4–6); this produces trisomy or monosomy of individual chromosomes at high frequency in multiple tissues[5,6]. Thus, although single chromosome gain or loss in cancer is rare and cytokinesis failure has probably occurred in some tumours that are highly aneuploid, current evidence indicates that nondisjunction produces near-diploid aneuploidy in primary mammalian cells, mice and humans.

A possible explanation for the correlation observed by Shi and King[1] is that one or more mitotic defects occurred that, in addition to causing nondisjunction, resulted in trapping of DNA in the cytokinetic furrow. Indeed, the authors observe chromatin bridging or lagging (events likely to lead to DNA trapping in the cleavage furrow) in more than half of cells that subsequently became binucleate. Although they conclude that there was no chromosome bridging in the remainder, some mitotic errors producing DNA within the cleavage furrow, including the strands of DNA that result from stretching of single kinetochores that are attached to microtubules from both poles, were below their sensitivity of detection. In our view, then, the most likely explanation for furrow regression and the subsequent binucleate, tetraploid state is not nondisjunction, but rather a DNA-damage response caused by chromatin breakage by the incoming cytokinetic furrow — as first suggested almost thirty years ago by the evidence in ref. 7.

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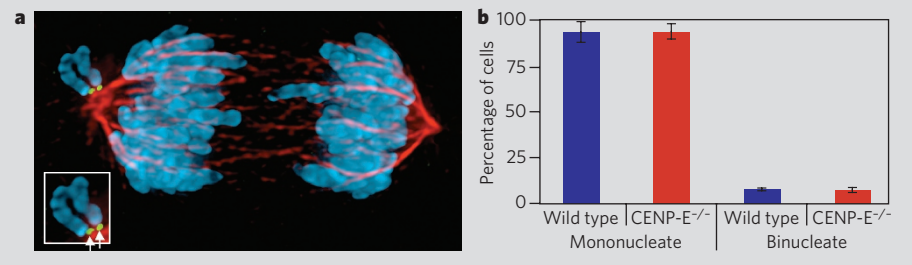

Figure 1 | Nondisjunction causes near-diploid aneuploidy, not cytokinesis failure, in primary cells. **a**, Anaphase chromosome distribution in a primary mouse fibroblast depleted of CENP-E (after CENP-E gene disruption mediated by Cre recombinase[2]). Inset, micrograph enlargement of one pair of duplicated, sister chromosomes undergoing nondisjunction. Arrows, centromeres. DNA, blue; tubulin, red; centromeres of unattached chromosomes (BUB1), green. **b**, Percentages of binucleate cells in wild-type and CENP-E-null populations; $n > 500$ cells from each of two independent experiments.

Methods. Chromosome spreads from lymphocytes from peripheral blood were prepared according to the Jackson Laboratory protocol (www.jax.org/cyto/blood_preps.html). The predicted percentage of binucleation in CENP-E-null cells was calculated as follows: if nondisjunction drives binucleation, the total number of binucleate progeny produced per division for CENP-E-null cells will be $0.25n$, where n is the number of mononucleated cells. As Shi and King have shown that binucleates survive (and either exhibit an extended interphase arrest or divide to produce daughters that are, in some cases, also binucleate)[1], and as long-term survival occurs in binucleate cells produced by inhibiting cytokinesis[8–10], most binucleates should survive for at least the length of one cell cycle. The total number of cells at the next division then is $0.25n + 2 \times 0.75n$. The steady-state proportion of binucleates generated by nondisjunction would thus be $0.25n/(0.25n + 2 \times 0.75n)$, or 14%, plus the 7% rate derived from other mechanisms in the parental wild-type cells, yielding an overall 21% binucleates, if nondisjunction yields binucleation.

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Shi & King reply

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Weaver *et al.* report[1] that chromosome nondisjunction induced by knockout of the mitosis-specific motor protein CENP-E does not increase the rate of binucleation in mouse fibroblasts and conclude that nondisjunction is therefore not coupled to furrow regression. However, they have not yet excluded alternative interpretations of their results, which makes it hard to compare them directly to our findings. Critical confounding issues include analysis of different cell types and the use of a specific gene deletion, for which corresponding naturally occurring mutations have not been described.

First, the differences could be explained by cell-type-specific differences in cytokinesis regulation. We evaluated three human epithelial cell lines[2], but the mouse fibroblasts examined by Weaver *et al.* may generate greater traction forces[3] that could promote cytokinesis completion through cell tearing[4], rather than by the targeted delivery and fusion of vesicles near the midbody[5]. Furrow regression occurs many hours after mitosis in HeLa cells[2] and, during the intervening period, cells remain connected by a thin cytoplasmic bridge. In fibroblasts, increased traction forces could break this bridge, uncoupling chromosome nondisjunction from furrow regression. Analysis of cytokinesis completion in CENP-$E^{+/+}$ and CENP-$E^{-/-}$ mouse fibroblasts by time-lapse imaging is necessary to exclude this possibility. Furthermore, it is essential that the CENP-E removal experiment be repeated in epithelial cells, which give rise to most human cancers.

Second, conclusions based solely on the analysis of CENP-$E^{-/-}$ cells is complicated by the fact that CENP-E itself (and its binding partner, BubR1) may be involved in coupling chromosome nondisjunction and cytokinesis completion. CENP-E is required for efficient congression of mono-orientated chromosomes to the metaphase plate[6] and for spindle checkpoint signalling[7]. Following anaphase, CENP-E localizes to the midbody[8] where it may negatively regulate cytokinesis completion[9]. Thus, given the many important functions of CENP-E in mitosis, CENP-E could act directly in coupling nondisjunction to cytokinesis completion, explaining why CENP-E removal would uncouple these two processes.

Third, nondisjunction in CENP-$E^{-/-}$ cells is unlike spontaneous nondisjunction. Nondisjunction induced by loss of CENP-E results from cells that enter anaphase with chromosomes remaining at the poles[10]. In contrast, we identified 17 cells that underwent nondisjunction, yet only two cells showed defects in chromosome congression[2]. Spontaneous nondisjunction therefore seems to be a different process from nondisjunction occurring in CENP-$E^{-/-}$ cells, providing another potential explanation for differences in the subsequent regulation of cytokinesis. The causes of spontaneous nondisjunction are poorly understood, but our time-lapse analysis indicates that perturbation of CENP-E function is unlikely to be among them.

Weaver *et al.* suggest that furrow regression is due to the presence of DNA in the cleavage furrow[1], but this possibility is inconsistent with our data: of 395 cells that completed cytokinesis, 58 showed chromosome bridging or lagging, indicating that these defects are not sufficient to induce furrow regression[2]. Furthermore, 47% of cells that became binucleated showed no bridging or lagging, indicating that these defects are not required for furrow regression. Although thin strands of trapped chromatin may be beyond our limit of detection, these cells also showed no evidence of micronuclei or disrupted interphase nuclear architecture, which might arise as a result of such defects. In contrast, cytokinesis failure has been reported only in cells showing gross distortions in nuclear architecture, with teardrop-shaped nuclei connected to one another by a large chromatin bridge[11]. We observed defects of this magnitude in only 11% of cells that became binucleated. Thus, although bridging and lagging may be associated with binucleation in a fraction of cells, we conclude that other mechanisms must exist to link nondisjunction and furrow regression in the remaining cells.

In budding yeast, there is a pathway that delays cytokinesis completion to prevent damage to chromosomes near the division site[12]. Although this mechanism has not been demonstrated in animal cells, our results indicate that such a pathway could possibly be activated more generally by spontaneous nondisjunction.

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