Aneuploidy and chromosome instability (CIN) are hallmarks of the majority of solid tumors, but the relationship between them is not well understood. In this issue, Thompson and Compton (Thompson, S.L., and D.A. Compton. 2008. Examining the link between chromosomal instability and aneuploidy in human cells. J. Cell. Biol. 180:665–672) investigate the mechanism of CIN in cancer cells and find that CIN arises primarily from defective kinetochore–spindle attachments that evade detection by the spindle checkpoint and persist into anaphase. They also explore the consequences of artificially elevating chromosome missegregation in otherwise karyotypically normal cells. Their finding that induced aneuploidy is rapidly selected against suggests that the persistence of aneuploid cells in tumors requires not only chromosome missegregation but also additional, as yet poorly defined events.

Aneuploidy describes the state of a cell containing an aberrant number of chromosomes, whereas CIN refers to an elevated rate of gain or loss of whole chromosomes per cell cycle. Both aneuploidy and CIN are phenotypes commonly observed in solid tumors. FISH analysis of aneuploid cancer cells cultured in vitro has been used to characterize the CIN phenotype and established that chromosome losses or gains occurred at \( > 10^{-2} \) per chromosome per cell cycle, which is 10–100 times greater than in karyotypically stable diploid cancers of the same histological subtype (Lengauer et al., 1997). Intratumoral heterogeneity in chromosomal numbers has also been reported (Furuya et al., 2000), implying that CIN occurs during tumor development in vivo. These studies imply a connection between the CIN phenotype and the aneuploidy of cancer cells. However, whether CIN is sufficient to generate and maintain the constantly changing spectrum of aneuploidy in cancer cells has not been determined, and the mechanisms that lead to the CIN phenotype remain poorly understood. Thompson and Compton address both of these issues using cultured cancer cells of different origins exhibiting the CIN phenotype as well as cancer cells with mismatch repair defects that do not demonstrate CIN and stably maintain diplody.

CIN is caused by an increased rate of chromosome missegregation, but the molecular mechanism underlying CIN remains unclear. Mutations in components of the spindle assembly checkpoint, which protects against chromosome missegregation by preventing progression to anaphase in the presence of chromosomes that have not yet connected properly to spindle microtubules (Musacchio and Salmon, 2007), have been identified in a small proportion of colon cancers (Cahill et al., 1998). Centrosome amplification has also been observed in some CIN cancer cell lines (Lingle et al., 2002). Recently, systematic sequencing of putative CIN phenotype–inducing genes, such as genes functioning at the kinetochore and involved in sister chromatid cohesion, has also been performed in different CIN cancers (Wang et al., 2004; Barber et al., 2008). These studies have implied a mutational origin to the CIN phenotype specifically caused by defects in physiological pathways that ensure accurate chromosome segregation.

Thompson and Compton (see p. 665 of this issue) first focus on the spindle assembly checkpoint and test whether there is a difference between CIN cancer cells and karyotypically normal cancer cells in the ability of the spindle checkpoint to detect chromosomes not yet attached to the spindle. In their original study implicating spindle checkpoint defects as the source of CIN, Cahill et al. (1998) showed that CIN cancer lines with mutations in the spindle checkpoint pathway fail to arrest when spindles are depolymerized. However, this conclusion is controversial. Tighe et al. (2001) concluded that CIN cancer cell lines undergo mitotic arrest in response to spindle damage and that the checkpoint pathway is functioning properly. Direct inhibition of spindle checkpoint genes has led to the idea that a loss of spindle checkpoint function is lethal to cells, but a partial defect in the checkpoint could underlie the CIN phenotype (Kops et al., 2005).

In the earlier contradictory studies, the checkpoint responses of CIN cells were characterized using fixed cell populations with nonphysiological extreme treatments (complete spindle depolymerization). Thompson and Compton (2008) addressed the mechanism of CIN by expressing GFP–histone H2B and following chromosome segregation at high resolution in individual cells. If the spindle checkpoint was weakened, cells were predicted to split their sister chromatids and enter anaphase before all chromosomes were connected to the spindle and aligned at the metaphase plate. This outcome was never observed in either CIN or diploid cancer cells. This finding lends further credence to the notion that the spindle checkpoint is functional enough to prevent premature sister chromatid separation in the CIN cancer cell lines.
These results suggest that chromosome missegregation leads to transient aneuploidy that is selected against in a population. Presumably, the genetic imbalances created by the aneuploidy impose a selective disadvantage. Consistent with this conclusion, a recent study in yeast showed that aneuploid yeast strains with one or more extra chromosomes were defective in cell cycle progression and exhibited slow growth, poor viability, increased glucose uptake, and increased sensitivity to conditions interfering with protein synthesis and protein folding as a result of the increase in protein production (Torres et al., 2007). However, the effect of aneuploidy on tumorigenesis may not be a simple positive or negative answer and may be influenced by the context and level of missegregation (Weaver and Cleveland, 2007).

Thompson and Compton (2008) also show that repeated rounds of induced chromosome missegregation in diploid cancer cells are not sufficient to generate persistent aneuploidy. It is possible that the number of consecutive chromosome missegregation events induced in this experiment was small compared with the number of generations needed for selection of a rare advantageous aneuploid event. Importantly, the results of the artificially induced missegregation experiments reveal that an additional event is required for the maintenance of aneuploid cells in a population (Fig. 1B). This tetraploidy hypothesis would be consistent with the near-tetraploid cells described in early stage cancers (Ganem et al., 2007). Tetraploidy could enhance the fitness of cells undergoing chromosome missegregation events through a buffering effect, allowing cells to survive until a crucial growth-enhancing or transforming mutation occurs. Other changes, such as mutations that reduce the efficacy of the apoptotic pathway, may also account for the survival of aneuploid CIN cancer cells. Finally, the assessment of fitness for the diploid cancer cells lines with artificially generated aneuploidy was performed in vitro under conditions in which appropriate

![Figure 1](image-url)

**Figure 1.** Induced chromosome missegregation in karyotypically stable diploid cell lines does not lead to persistent aneuploidy; another, as yet unknown change is required for persistent propagation of aneuploid cells. (A) The proportion of aneuploid cells drops over time after induced chromosome segregation in a karyotypically stable diploid cancer cell line but stays high in CIN cell lines. Dashed lines represent the basal level of aneuploidy in the diploid and CIN cell lines. (B) Model summarizing the key conclusion from the artificially induced missegregation experiments.
environmental cues may be missing or not limiting for proliferation. The situation may be quite different in vivo, and whether the conclusion obtained by Thompson and Compton (2008) will extend to in vivo tumorigenesis models will be important to address in the future.

The study highlighted here (Thompson and Compton, 2008) provides the most rigorous evidence to date against a weakened spindle checkpoint being the source of the CIN phenotype and sheds light on the link between CIN and aneuploidy. The use of such a direct approach in a controlled experimental system lends weight to the notion that diploidy is the favored state. In other words, the wages of CIN is death; whether this outcome arises from slow attrition as a result of reduced fitness in a population of proliferating cells or an active cell death mechanism remains unclear. The additional changes that allow CIN cancer cells to persistently generate and maintain a spectrum of aneuploidy will be critical to identify in future work.

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