# The wages of CIN

Karen W. Yuen[1,2] and Arshad Desai[1,2]

[1]Ludwig Institute for Cancer Research and [2]Department of Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA 92093

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 diploidy.

Correspondence to Karen W. Yuen: kayuen@ucsd.edu; or Arshad Desai: abdesai@ucsd.edu

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.


The Journal of Cell Biology, Vol. 180, No. 4, February 25, 2008 661–663
http://www.jcb.org/cgi/doi/10.1083/jcb.200801030

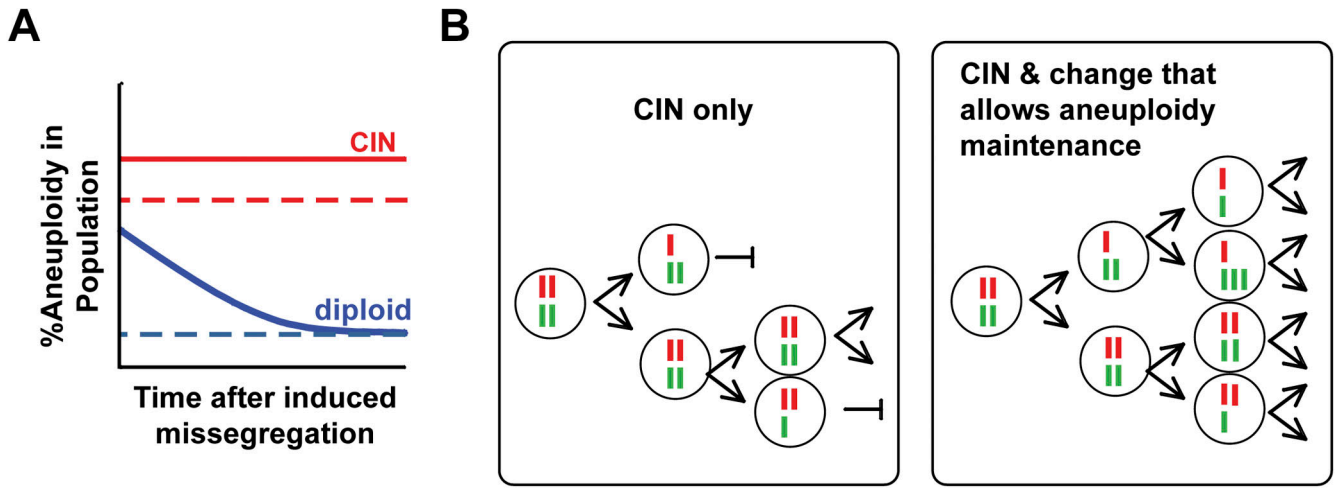


Figure 1. **Induced chromosome missegregation in karyotypically stable diploid cell lines does not lead to persistent aneuploidy; another, as yet unknown change is required for persistent propagation of aneuploid cells.** (A) The proportion of aneuploid cells drops over time after induced chromosome 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.

Thompson and Compton (2008) noticed in their live cell imaging that lagging chromatids in anaphase occurred at a higher frequency (24–75%) in CIN lines as compared with karyotypically normal cells (<10%). The number of lagging chromatids per anaphase in CIN cells is 3–14-fold higher than in karyotypically normal cells, a greater increase than would be expected from the increased modal number of chromosomes in the aneuploid CIN cell lines. Fixed cell analysis revealed that the lagging chromatids often have merotelic attachment, in which the single kinetochore of a sister chromatid attaches to microtubules emanating from opposite spindle poles. This type of improper microtubule–chromosome attachment escapes detection by the spindle assembly checkpoint and increases the likelihood of chromosome missegregation (Cimini et al., 2001). The reason for the increase in frequency of merotely in CIN cancer cell lines is not yet known. It is possible that defects in the early stages of spindle assembly, which are known to increase merotely (Cimini et al., 2001), are the underlying cause.

In addition to exploring the mechanism of CIN, Thompson and Compton (2008) artificially elevated chromosome missegregation rates in karyotypically normal cancer cells to test whether induced CIN causes persistent aneuploidy. Previous work had established that treatment with reversible spindle assembly–perturbing drugs followed by their washout correlated with increased merotelic attachments and chromosome missegregation (Cimini et al., 2001). The authors used this scheme to increase chromosome missegregation in both CIN cancer lines and diploid cancer cell lines. They then monitored the fate of the treated cells using FISH to count chromosomes. For the diploid cancer cell lines, the proportion of aneuploid cells increased 4 h to 2 d after the drug washout treatment (Fig. 1 A). The chromosome number among cells within individual clonal colonies varied, suggesting that multiple, independent chromosome missegregation events had occurred reminiscent of the CIN phenotype. However, aneuploidy in the diploid cancer cell lines disappeared from the population over time (Fig. 1 A). 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 CIN and aneuploid phenotypes in the population surviving the treatments. 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 elevated missegregation experiments reveal that an additional event is required for the maintenance of aneuploid cells in a population (Fig. 1 B). It has been suggested that aneuploid cancer cells can arise through an unstable tetraploid intermediate (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. This tetraploidy hypothesis would be consistent with the near-tetraploid cells described in early stage cancers (Ganem et al., 2007). 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

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.

Work in the Desai laboratory is supported by the Ludwig Institute for Cancer Research and grants from the National Institutes of Health and the Human Frontiers Science Program.

Submitted: 25 January 2008
Accepted: 30 January 2008

## References

Barber, T., K. McManus, K. Yuen, M. Reis, G. Parmigiani, D. Shen, I. Barrett, Y. Nouhi, F. Spencer, S. Markowitz, et al. 2008. Chromatid cohesion defects may underlie chromosome instability in human colorectal cancers. *Proc. Natl. Acad. Sci. USA.* In press.

Cahill, D.P., C. Lengauer, J. Yu, G.J. Riggins, J.K. Willson, S.D. Markowitz, K.W. Kinzler, and B. Vogelstein. 1998. Mutations of mitotic checkpoint genes in human cancers. *Nature.* 392:300–303.

Cimini, D., B. Howell, P. Maddox, A. Khodjakov, F. Degrassi, and E.D. Salmon. 2001. Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic mammalian tissue cells. *J. Cell Biol.* 153:517–527.

Furuya, T., T. Uchiyama, T. Murakami, A. Adachi, S. Kawauchi, A. Oga, T. Hirano, and K. Sasaki. 2000. Relationship between chromosomal instability and intratumoral regional DNA ploidy heterogeneity in primary gastric cancers. *Clin. Cancer Res.* 6:2815–2820.

Ganem, N.J., Z. Storchova, and D. Pellman. 2007. Tetraploidy, aneuploidy and cancer. *Curr. Opin. Genet. Dev.* 17:157–162.

Kops, G.J., B.A. Weaver, and D.W. Cleveland. 2005. On the road to cancer: aneuploidy and the mitotic checkpoint. *Nat. Rev. Cancer.* 5:773–785.

Lengauer, C., K.W. Kinzler, and B. Vogelstein. 1997. Genetic instability in colorectal cancers. *Nature.* 386:623–627.

Lingle, W.L., S.L. Barrett, V.C. Negron, A.B. D'Assoro, K. Boeneman, W. Liu, C.M. Whitehead, C. Reynolds, and J.L. Salisbury. 2002. Centrosome amplification drives chromosomal instability in breast tumor development. *Proc. Natl. Acad. Sci. USA.* 99:1978–1983.

Musacchio, A., and E.D. Salmon. 2007. The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol.* 8:379–393.

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.

Tighe, A., V.L. Johnson, M. Albertella, and S.S. Taylor. 2001. Aneuploid colon cancer cells have a robust spindle checkpoint. *EMBO Rep.* 2:609–614.

Torres, E.M., T. Sokolsky, C.M. Tucker, L.Y. Chan, M. Boselli, M.J. Dunham, and A. Amon. 2007. Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science.* 317:916–924.

Wang, Z., J.M. Cummins, D. Shen, D.P. Cahill, P.V. Jallepalli, T.L. Wang, D.W. Parsons, G. Traverso, M. Awad, N. Silliman, et al. 2004. Three classes of genes mutated in colorectal cancers with chromosomal instability. *Cancer Res.* 64:2998–3001.

Weaver, B.A., and D.W. Cleveland. 2007. Aneuploidy: instigator and inhibitor of tumorigenesis. *Cancer Res.* 67:10103–10105.