Response to Comment on "Cell Type Regulates Selective Segregation of Mouse Chromosome 7 DNA Strands in Mitosis"

Amar J. S. Klar[1]* and Athanasios Armakolas[2]

To explain how all chromosome recombinants can become homozygous for a marker located distal to the crossover point, we proposed that mitotic recombination must be restricted to two specific chromatids and that the selective chromatid segregation process follows recombination. We refute Haber's contention that our results can be explained by the conventional X-segregation process if recombination of all possible combinations of chromatids is considered.

Haber (*1*) reiterates the explanation of Beumer *et al*. (*2*) in the *Drosophila* system and of Liu *et al*. (*3*) for how *Cre/loxP*-induced chromosome 7 mitotic recombinants always become homozygous by the standard X-segregation process occurring in mouse embryonic stem (ES) cells. The X segregation is so defined whenever recombined chromatids segregate away from each other to opposite poles of the mitotic spindle, resulting in homozygosis of both daughter cells. Accordingly, crossover in any pair of nonsister chromatids would produce homozygous recombinants. This explanation (*1*) is suggested to challenge our model, which purportedly considered recombination of only two, and not all possible, combinations of nonsister chromatids (*4*, *5*). We disagree with all of Haber's points.

The descriptive terms X and Z segregation were originally coined to describe homozygous and heterozygous recombinants, respectively. These terms describe only the segregation outcome and are not meant to include the mechanism of how recombined chromatids preferentially segregate to one pole or the other. In general, both X and Z types of recombinants are found in a culture, so there is no compelling reason to question their origin. Unexpectedly, however, in mouse ES (*3*) and endoderm cells, all recombinants show only the X pattern, and only the Z pattern is found in neuroectoderm cells (*5*). In this context, Beumer *et al*. (*2*) previously hypothesized that sister chromatid cohesion may promote X segregation in *Drosophila*. Even if this explanation holds for the *Drosophila* system, in which homologs remain paired, such a mechanism is not likely to operate in the mouse system, in which extensive chromosome pairing has not been observed. However, the issue remains unresolved, and alternative explanations should be considered.

Haber comments that if recombination occurs in all combinations of nonsister chromatids as is normally expected, and that if recombined chromatids segregate by X segregation, that there will be no biased segregation of the "older" "Watson" versus older "Crick" strand–containing chromatids. He suggests that, because we did not consider the possibility of all chromatids recombining, our model is invalid. In fact, we had logically considered recombination involving all combinations of chromatids as explained in the legend to figure 1 in (*5*). However, to achieve only X segregation or only Z segregation, our hypothesis deliberately stipulated that recombination must occur in two specific chromatids only. As cells have the capability to segregate nonrandomly, chromatids must be distinct from each other. We hypothesize that they are distinguished inherently on the basis of the older (Watson) versus newer (Crick) DNA strands that they inherit. Several results presented in our paper (*5*) and in the Liu *et al*. (*3*) study are consistent with our hypothesis, and we therefore concluded that the pattern is chromosome-specific and changes with cell type.

Haber points out that there is no precedent to support the idea that Cre-recombinase favors recombination of one chromatid over the other. We are not aware of any study designed to test this hypothesis; also, new ideas, by definition, do not have precedence. Moreover, we have proposed that, rather than being a property of the recombination system itself, restricted chromatid recombination is a manifestation of the selective strand-segregation mechanism operating in cells where recombination does not normally occur. Furthermore, preferential strand segregation was originally conceived as a theoretical mechanism for nonrandom segregation of sister chromatids in general mitosis (*6*). The recombination system provides a specific test of the model, and the X-segregation result provides supportive evidence for it.

Given the potential importance of the homozygous result for cellular biology, it is premature to think that the reason for preferential X segregation is already understood. Meanwhile, how can we distinguish the conventional X-segregation explanation (*1*) from that of our model? If it can be shown experimentally that all chromatids recombine equally well, our model will be invalidated. If it can be shown that heterozygous (Z type) recombinants we found in lineages other than ES and endoderm type (*5*) result from G2-phase recombination, in which recombined chromatids must cosegregate to the same pole/cell, then the conventional X-segregation explanation would be invalidated. Because ours is the newest hypothesis that attempts to explain the remarkable result of biased chromatid segregation, it should be further analyzed. Such studies should be extended to other chromosomes and to other organisms, using other approaches, not only to define the mechanisms of the selective- versus random-chromatid segregation phenomenon but also to further scrutinize the validity and generality of our model.

[1]Laboratory of Gene Expression and Chromosome Biology, National Cancer Institute at Frederick, P.O. Box B, Frederick, MD 21702–1201, USA. [2]Hippokrateion Hospital of Athens, Laboratory of Surgical Research, Vassilisis Sofias 114, Athens 11527, Greece.

*To whom correspondence should be addressed. E-mail: klar@ncifcrf.gov

References and Notes

1. J. E. Haber, *Science* **313**, 1045 (2006); www.sciencemag.org/cgi/content/full/313/5790/1045b.
2. K. J. Beumer, S. Pimpinelli, K. G. Golic, *Genetics* **150**, 173 (1998).
3. P. Liu, N. A. Jenkins, N. G. Copeland, *Nat. Genet.* **30**, 66 (2002).
4. A. J. S. Klar, *Genetics* **167**, 1833 (2004).
5. A. Armakolas, A. J. S. Klar, *Science* **311**, 1146 (2006).
6. A. J. S. Klar, *Trends Genet.* **10**, 392 (1994).
7. The Intramural Research Program of NCI supported this research.

10 April 2006; accepted 25 July 2006
10.1126/science.1128552

Response to Comment on "Cell Type Regulates Selective Segregation of Mouse Chromosome 7 DNA Strands in Mitosis"

Amar J. S. Klar and Athanasios Armakolas

Science **313** (5790), 1045.
DOI: 10.1126/science.1128552