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

James E. Haber

Armakolas and Klar (Reports, 24 February 2006, p. 1146) suggested that segregation of mouse chromosome 7, after induction of a site-specific crossover between homologous chromosomes, is driven by a preferential inheritance of the old Watson and the old Crick DNA strands. However, this interpretation only considered half of the possible outcomes. The conjecture fails when all possible outcomes are examined.

When Copeland and colleagues (1) used site-specific recombination to create crossovers near the centromere of chromosome 7 in mouse embryonic stem (ES) cells, they made the observation that es-

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sentially all of the recombinants segregated such that the reciprocal crossover products segregated to opposite sister cells. Consequently, the cells became homozygous for all the markers distal to the point of exchange (designated X segregation) (Fig. 1). Recently Armakolas and Klar (2) repeated these experiments and offered a more striking interpretation of these data. Specifically, they proposed that chromosome segregation is

driven by a preferential inheritance of the old strands of DNA so that one sister cell inherits the old Watson (W) strands and the other inherits the old Crick (C) strands (WC′ WC′ or W′C W′C, where W′ and C′ indicate the more recently replicated new strands). This arrangement is abbreviated WW::CC. The model apparently assumes that the key sequences governing segregation are those centromere-proximal to the site of crossover, as the more distal sequences will have exchanged old W or C strands for new (Fig. 1A). However, this model is apparently based on an evaluation of only half of the possible outcomes of crossover in the G2 phase of the cell cycle; the other half of the events do not support the proposed interpretation.

Armakolas and Klar's figure 1A in (2), reproduced in a slightly different form in Fig. 1, illustrates an exchange between chromatids 2 and 3 to generate an HGPRT⁺ recombinant, which is recovered by selection (Fig. 1A). The X-segregation outcomes from this arrangement yield chromosomes that seem to obey the WW::CC pattern of segregation. However, it is just as likely that chromatid 3 will recombine with chromatid 1 as with chromatid 2. If 3 × 1 exchange occurs (Fig. 1B), X segregation of the
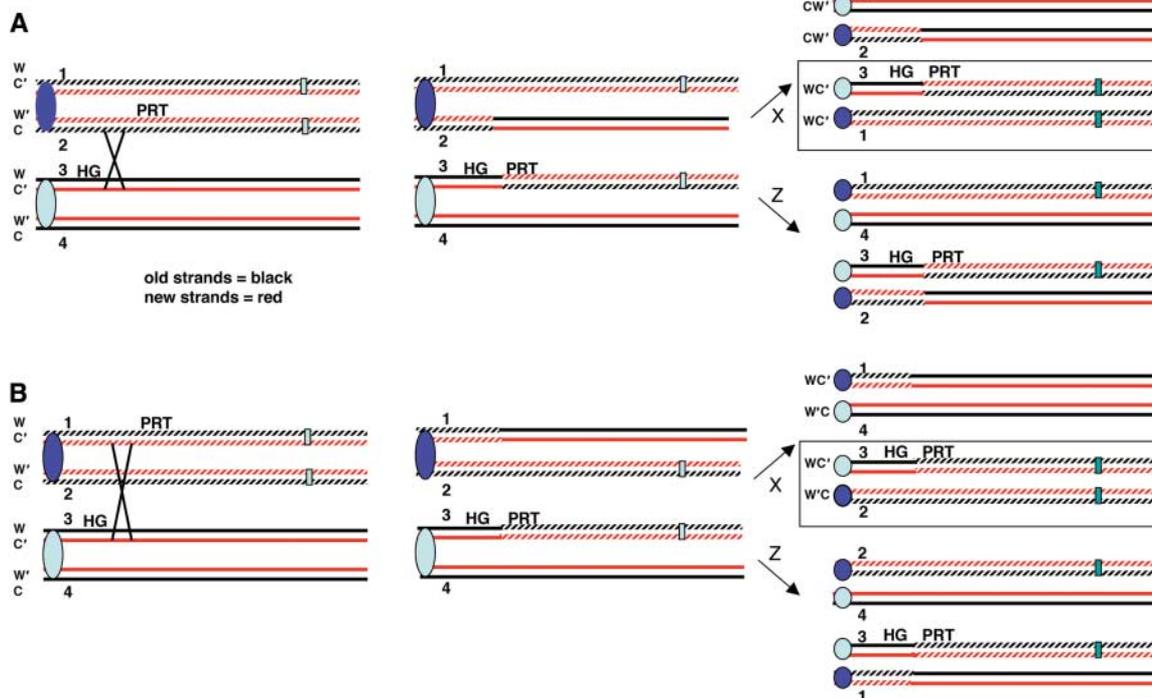
Fig. 1. Consequences of reciprocal crossover and biased strand segregation. Site-specific recombination creates a reciprocal recombinant, scored as HGPRT⁺ (1). Only HGPRT⁺ cells are recovered and are relevant in this analysis. Biased chromatid segregation, such that the reciprocally recombined chromosomes segregate away from each other (X) or with each other (Z), is shown. Old strands of DNA are shown in black for the two homologous chromosomes (distinguished by solid and dashed lines), and new strands are drawn in red. Strands oriented 5′ to 3′ (Watson) are designated W, and the newer strands are designated W′, whereas 3′ to 5′ strands are designated Crick (C or C′). (A) Recombination between chromatids 2 and 3 yields X segregants in which the DNA strands near centromere regions (circles) are either W′C W′C or WC′ WC′ (that is, the two oldest W and the oldest C strands cosegregate). The box highlights the HGPRT⁺ recombinant that is selected. In addition, X segregation results in more distal markers (e.g., the blue rectangle) becoming homozygous in each daughter cell. (B) A similar recombination event between chromatids 1 and 3 yields HGPRT⁺ recombinants that are still homozygous for the distal marker, but regions near the centromeres are no longer W′C and WC′ WC′. The HGPRT⁺ recombinant expected for X segregation is boxed.

crossover chromatids to opposite poles will still produce cells homozygous for paternal or maternal distal markers, consistent with Copeland's conclusion (*1*), but there will no longer be WW::CC segregation of centromere-proximal regions. The same considerations apply if crossovers involve chromatid 4 and either chromatid 1 or 2. There is no precedent to support an idea that site-specific recombination is more likely to promote recombination with one chromatid over the other. Thus, the idea that there will be age-directed segregation of chromosome 7 chromatids is not supported when one examines all possible outcomes of a single exchange event in the G2 phase of the cell cycle.

Armakolas and Klar also found that in differentiated cells containing the same chromosome 7 construct and derived from the original ES cells, the outcomes were different. Most striking, whereas endodermal cells yielded X segregation, neuroectodermal cells yielded almost exclusively Z type. This difference could

be attributed to a change in the time of recombination from G2 to G1, because if the exchanges between homologous chromosomes occur before DNA replication, only Z types will be recovered (*1*, *2*). It is interesting to note that when Liu *et al.* (*1*) carried out analogous Cre-mediated exchanges at sites on chromosome 11, the rate of recombination was lower by a factor of 20, and both X- and Z-type segregations were found. In this regard, it may be relevant that the recombination rate in neuro-ectoderm cells was lower by a factor of 25 than in endodermal cells; it is possible that the low-frequency events occur predominantly in G1. It would be interesting to know whether the pattern of Cre recombinase expression differs in different cell types or whether the recombining sites exhibit different accessibility in the G1 and G2 stages of the cell cycle.

The suggestion that chromosomes segregate based on their replication history is of great interest. The reported experiments examine only

cases where there has been a site-specific cross-over to follow strand segregation. It might be possible to examine all cells in a population by the method of chromosome orientation–fluorescence in situ hybridization (CO-FISH) (*3*), in which strand-specific probes hybridize only to the older strands of chromosomes that had not incorporated bromodeoxyuridine (BrdU). Such an experiment should be possible if cells were allowed to proceed through mitosis so that each daughter cell, each containing one BrdU-labeled strand on each homologous chromosome, could be probed with strand-specific probes to see if there is WW::CC segregation.

References

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