INVITED EDITORIAL
X-Chromosome Inactivation Spreads Itself: Effects in Autosomes

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Despite recent major advances, the mechanism of X-chromosome inactivation remains largely a mystery. X;autosome translocations have been a source of insight into this mechanism since the original discovery of the phenomenon. The first evidence for the existence of an X-inactivation center came from the spread of X inactivation from the X chromosome into the attached autosome—but from only one of the two segments into which the X chromosome was broken, putatively that bearing the X-inactivation center (Russell 1963). Now, important further insight is provided in the report, by White et al. (1998) in this issue of the *Journal,* of a human female with an unbalanced X;autosome translocation involving the X chromosome and the autosome 4q.

The important new feature is that these authors studied the activity of 20 genes and expressed sequence tags (ESTs) in the autosomal segment attached to the X chromosome. In previous work with translocations, it had been possible to study only one or a few genes—and not at the molecular level. White et al. made somatic-cell hybrids carrying only the inactive translocated X chromosome from this female and have cloned the cells. They found that, as assessed by reverse transcription–PCR, 14 of the 20 autosomal genes and ESTs were totally inactive but that the other 6 were clearly expressed; thus, 14 genes and ESTs had undergone X inactivation, but 6 had escaped. Five of the 6 expressed genes were interstitial, with inactivated genes on both sides, and it appeared that the inactivation had spread almost or entirely to the end of the attached segment of 4q, a distance of 100 Mb. Thus, those genes that had escaped inactivation had either resisted the original inactivation signal or undergone reactivation as a result of failure of the mechanism to maintain the inactive state. On the X chromosome itself in humans, some genes escape inactivation (reviewed in Disteche 1995), but the proportion is considerably lower than the 6/20 genes found for this translocation; in the mouse X chromosome, even fewer genes escape silencing. Thus, White et al. conclude that autosomal genes are more likely than X-linked genes to escape inactivation. This provides evidence at a molecular level that X-chromosomal and autosomal chromatin somehow respond differently to X-inactivation signals. X-chromosomal material is clearly not essential for X inactivation to occur, but it somehow provides a more favorable basis for it.

This finding, in itself, is not new. It was already clear, from early work on mouse X;autosome translocations, that inactivation of attached autosomal segments was incomplete. Inactivation of autosomal coat-color genes translocated to the X chromosome was observed as variegated patches in the coat. Those color genes at greater distances from the translocation breakpoint were less likely to be inactivated (Russell 1963, 1983; Russell and Montgomery 1970). Animals could have whole patches in which the nearer of two genes was inactive but in which the more distal one was not. This implies that, at an early embryonic stage, inactivation had traveled different distances in different cells and that the distance traveled had then remained constant through further cell divisions. A complication, however, was Cattanach's (1974) demonstration that the albino gene, when translocated to the X chromosome, could undergo reactivation even at the adult stage. Thus, observed escape from X inactivation can be either primary or due to reactivation.

Recent work has provided insight into how the travel of X inactivation is brought about. The *XIST*/*Xist* gene, located at the X-inactivation center, encodes a large untranslated RNA that coats the inactive X chromosome (Clemson et al. 1996; Panning and Jaenisch 1996). By knockouts and transgenes, the *Xist* gene has been shown to be necessary and sufficient for X inactivation (Lee et al. 1996; Penny et al. 1996; Herzing et al. 1997; Marahrens et al. 1997). Lee and Jaenisch (1997) used an *Xist* YAC transgene inserted near the centromere of mouse chromosome 12 to study the effect of *Xist* on the autosome. The *Xist* RNA coated chromosome 12 as it normally coats the X chromosome. In addition, chro-

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mosome 12 was late replicating and hypoacetylated, as is a normal inactive X chromosome. Furthermore, three of four chromosome 12 genes studied were not expressed. Thus, *Xist* RNA can travel for long distances in autosomes and can bring about long-range spreading of X inactivation. However, the most distal part of chromosome 12 was not hypoacetylated in all cells, and, of the four genes whose expression was studied, the most distal one was partly expressed. Thus, in this case it appears that the effects of the *Xist* RNA were not reaching the most distal part of the autosome. Like the early translocation work, this suggests that there is something about X-chromosomal material that favors the spread of *Xist* RNA or its effects.

In White et al.'s work, restriction of travel of *Xist* RNA does not appear to be the problem, since all except one of the noninactivated genes were interstitial. As the authors point out, this still leaves several possible explanations of the results. The way in which the coating of chromatin with *XIST*/*Xist* RNA brings about inactivation is not understood. Perhaps, in this case, it is failing to make effective contact at critical points. Furthermore, it is not clear whether the failure of inactivation is at the level of individual genes or at the level of chromosomal domains. Goldman (Gartler et al. 1992) has suggested that X inactivation occurs at the level of chromosomal domains of ~100 kb, each domain having a signal sequence. Autosomal domains might lack such signal sequences, leading to their escape from inactivation. It is also possible that the individual noninactivated genes have "boundary elements" that protect them from the effects of *XIST* RNA. Yet another possibility is that the genes are initially inactivated but are later reactivated, as has been shown, by Cattanach (1974), for the albino gene. The maintenance of the inactive state is thought to be brought about by a combination of feedback mechanisms involving (*a*) asynchronous replication of the inactive X chromosome and (*b*) differential methylation of CpG islands in promoters of X-linked genes (reviewed by Riggs 1990; Lyon 1996). Whether *XIST*/*Xist* RNA has any role in maintenance of inactivation is not clear, since the inactive state can be maintained in cells that have lost the *XIST* gene after initiation of X inactivation (Brown and Willard 1994; Rack et al. 1994). It is possible that the mechanisms leading to late replication and differential methylation are less efficient in autosomal chromatin, although it should be noted that, in the mouse, reactivation has been reported for X-linked genes (Wareham et al. 1987), as well as for the autosomal albino gene.

The material provided by this somatic-cell hybrid, although very valuable, does have certain limitations. The hybrid is a single-cell clone, and hence it is not clear whether all cells from the individual studied by White et al. had the same sets of genes active and inactive. In mice, as has been mentioned above, specific color genes may be active or inactive in different cells of the same animal, and the degree of inactivation of the same gene may vary from one translocation to another. Rastan (1983), using the Kanda staining typical of the inactive X chromosome, to study mouse X;autosome translocations, found that the extent of spread of this property into the autosome differed from cell to cell, including one translocation in which the non–Kanda-staining region was interstitial. Even if all cells show the same inactivation pattern in this female, it could be that other patterns were originally formed but were eliminated by cell selection during development, since the pattern seen, with almost all of the attached 4q segment inactive, restores genetic balance and thus could be favorably selected.

Despite the limitations, this material is likely to prove very valuable in providing further insight into the mechanism of X inactivation. On the basis of the early translocation work, it has been suggested that there are "way stations" or "boosters" on the X chromosome that promote the spread of X inactivation (Gartler and Riggs 1983; Riggs 1990). Elsewhere, I have proposed (Lyon 1998) that the function of such boosters might be provided by repetitive LINE elements. The distribution of these in the genome is appropriate, in that they are found dispersed in all autosomes, but the entire mouse X chromosome and the pericentromeric region of the human X chromosome are particularly rich in them (Korenberg and Rykowski 1988; Boyle et al. 1990). Long interspersed DNA sequence elements (LINEs) are found preferentially in dark G-bands, and the suggestion has been that, where LINEs are dense, *Xist* RNA makes contact and its travel is boosted and that, where LINEs are sparse, it loses contact and becomes dispersed and/or degraded. Furthermore, it has been suggested that contact of *Xist* RNA with LINE elements might bring about repeat-induced gene silencing (Wolffe 1997), embracing the interposed unique-sequence DNA of the X chromosome as well as the LINEs themselves. Since the mechanism of repeat-induced gene silencing is not known, it is not clear whether *Xist* RNA is in fact a good candidate to bring it about. Further studies of White et al.'s somatic-cell hybrids may provide evidence for or against these suggestions and surely will answer other questions concerning the mechanism of X inactivation and the function of *XIST*/*Xist* RNA.

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