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INVITED EDITORIAL
The Great Escape

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The existence of genes that escape X inactivation—that is, genes that are expressed from both the active and the inactive X chromosomes in somatic cells—was first suspected on the basis of the abnormal phenotype of Turner syndrome in individuals with a single X chromosome (Ferguson-Smith 1965). Escape from X inactivation was first demonstrated in humans by following XG blood-group polymorphisms (Fialkow 1970). From then on, various methodologies were developed to demonstrate escape. Measurements of steroid sulfatase (gene *STS*) activity in cell clones from patients heterozygous for *STS* deficiency demonstrated the existence of genes that escape inactivation and are located outside the pseudoautosomal region. This was seen as *STS* expression in all clones, regardless of the X chromosome that they carried (Shapiro et al. 1979).

Somatic cell–hybrid systems have been the method of choice for most analyses of the X-inactivation status of human genes. Mohandas et al. (1980) were the first to exploit interspecific hybrid cell lines resulting from fusion of human and mouse cells, to confirm that the *STS* gene is expressed from the inactive X chromosome. This methodology has been complemented by a second type of assay in some cases: for example, the *ZFX* gene displayed both positive expression in interspecific hybrid lines and increased expression in cell lines containing increasing numbers of X chromosomes (Schneider-Gädicke et al. 1989). Complementation of a mouse temperature-sensitive cell-cycle mutant was another novel—but gene limited—method to demonstrate that the ubiquitin-activating enzyme (*UBE1*) gene also escapes X inactivation (Brown and Willard 1989).

The availability of many DNA sequences from the X chromosome allowed reverse-transcription–PCR (RT-PCR) assays for X-linked gene expression on somatic cell–hybrid lines retaining the inactive human X chromosome on a rodent background. Of the genes found to escape X inactivation by this method, the most famous is *XIST* (Brown et al. 1991). *XIST* is expressed only from the inactive X chromosome and is essential for initiation of X inactivation (Penny et al. 1996). Furthemore, insertions of transgenes containing *XIST* initiated dosage compensation of the autosomes into which they were inserted (Hertzing et al. 1997; Lee and Jaenish 1997). *XIST* RNA decorates the inactive X chromosome, suggesting that this RNA product may be intimately involved in the epigenetic changes associated with X inactivation (Clemson et al. 1996).

The paper by Brown et al. (1997) in this issue of the *Journal* reports further systematic examination of the X-inactivation status of 33 genes by RT-PCR in a series of eight somatic cell–hybrid lines retaining either an active or an inactive human X chromosome on a rodent background. The X-inactivation status of many of the genes examined had already been known, and these results were confirmed and extended in this analysis. New genes that escape also were found. One of the most interesting findings by Brown et al. was the high proportion of genes that escape X inactivation: even after eliminating bias in the choice of genes to examine, the authors estimate that 4/17, or perhaps as many as a quarter, of X-linked genes may escape inactivation.

The other unexpected finding was that not all the hybrid cell lines displayed consistent results in terms of expression of a given gene. Indeed, 5/33 genes examined showed heterogeneous expression in the hybrid cell lines. Although in one case heterogeneous expression was evidenced by the absence of expression in a cell line retaining the active X chromosome, in four other instances heterogeneous expression was evident in the hybrid cell lines retaining the inactive X chromosome. These results could be interpreted as instability of inactivation in interspecific hybrid cell lines, in which induced reactivation occurs at relatively high frequency (Mohandas et al. 1981). However, spontaneous reactivation is rare in hybrid cell lines retaining the inactive human X chromosome (Kahan and DeMars 1975; see review in Gartler and Goldman 1994), and thus artifacts of cell culture are an unlikely explanation for the heterogeneity of expression observed by Brown et al. Stability of X inactivation also has been observed in cultured diploid cells with a very rare example of reactivation (Migeon et al. 1982).

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An intriguing alternative explanation for the heterogeneity of expression observed by Brown et al. is that there is heterogeneity of expression in the somatic cells used in the original cell fusions to derive the hybrid cell lines—and that such heterogeneity is likely to exist in vivo. Little is known of the stability of expression of X-linked genes in individual cells in vivo. Reactivation does occur normally in female germ cells (Gartler et al. 1975). Age-related reactivation of the X-linked gene for ornithine transcarbamylase (*Otc*) has been observed in individual mouse cells in vivo (Wareham et al. 1987), although no such reactivation was observed for other genes (see review in Gartler and Goldman 1994).

Heterogeneity of expression also may occur during development. For example, an alpha-fetoprotein transgene has been reported to escape X inactivation in extraembryonic membranes but not in the embryo per se (Krumlauf et al. 1986), and a human collagen transgene has been reported to escape X inactivation in a subset of embryonic cells, whereas other cells showed complete inactivation (Wu et al. 1992). Tan et al. (1993) followed expression of a reporter transgene and observed variable timing of X inactivation in different mouse tissues. This variability was not observed, however, in the study of two endogeneous genes that are subject to X inactivation (Lebon et al. 1995). This suggests that different genes behave differently in terms of timing and stability of inactivation. Our own studies of the allelic expression of *Smcx,* a gene that escapes X inactivation in adult mice (Agulnik et al. 1994; Wu et al. 1994), showed great variability of expression from the inactive X chromosome in individual embryonic cells, including cells with complete inactivation or complete escape, observed during development (P. Lingenfelter and C. Disteche, unpublished results). Both Carrel et al. (1996) and Sheardown et al. (1996) reported some differences in *Smcx* levels of escape during mouse development, but the cell-to-cell variability was not examined in those studies.

That humans may have many genes that escape X inactivation is in sharp contrast to the mouse, where few genes have been found that escape X inactivation (see review in Disteche 1995). The paucity of genes that escape X inactivation in mouse may reflect either the smaller number of genes examined or, perhaps, methodological differences. The methodology to assay mouse genes is based on in vivo analysis of expression in X;autosome translocation–carrier mice (Adler et al. 1991; Ashworth et al. 1991), a system that differs from the human in vitro systems described above. However, stable inactivation of X-linked genes has been demonstrated in transformed mouse cell lines and in interspecific hybrid lines (Bressler et al. 1993; Salido et al. 1993). The difference between human and mouse, with respect to the number of genes that escape X inactivation, may explain the difference in severity of the phenotype of monosomy X, between the species (see review in Zinn et al. 1993). Indeed, humans with Turner syndrome show both greatly reduced viability in utero and a number of phenotypic abnormalities that likely result from haploinsufficiency of X-linked genes normally expressed in two copies in females. Such abnormalities are not found in XO mice, who show only a slight reduction in developmental viability and in fertility (Banzai et al. 1995).

The mechanisms of escape from X inactivation are still unknown. Escape could occur at the onset of X inactivation, or it could result from reactivation. Our data on *Smcx* expression during development are consistent with reactivation, since *Smcx* is susceptible to complete inactivation in some cells (P. Lingenfelter and C. Disteche, unpublished results). Alternatively, a process of cell selection may occur. Progressive reactivation may result from loss of epigenetic modifications that control gene expression.

Brown et al. (1997) suggest that their data may indicate different levels of epigenetic control of X inactivation. One can imagine that some X-linked genes are more stably inactivated because of more stringent epigenetic changes such as the extent of methylation, histone deacetylation, chromatin structure modifications, and replication timing. Other genes may lack some of these controls and thus may be more susceptible to reactivation. Escape from X inactivation can be partial, with lower expression from the inactive X chromosome (Migeon et al. 1982), possibly reflecting persistence of some of the controls of gene expression. Comparison of the promoter sequences of the human *ZFX* and mouse *Zfx* genes has shown that they are remarkably similar, at least in terms of sequence, and thus has yielded no immediate clue to explain why this conserved gene escapes inactivation in human but not in mouse (Luoh et al. 1995). One possibility is that there are regional controls of expression, such as chromatin-domain controls, as suggested by Goldman et al. (1987) and Hansen et al. (1996), which would be consistent with the clustering of genes that escape X inactivation. Additional controls would be at the level of individual genes, which would explain the close proximity of genes with opposite X-inactivation patterns (Miller et al. 1995).

Strong selective pressure also may work to keep X-linked genes in one given state of activity. For some genes, dosage may be critical, and thus inactivation would be maintained in all cells, either by stringent controls as described above or by the elimination of cells that undergo reactivation. Conversely, escape from inactivation may occur to maintain equal dosage in females as compared with males with a Y-linked homologue of the gene that escapes X inactivation. Many of the genes that escape inactivation have Y homologues. So far, only one of these homologues, *RPS4Y,* which encodes a ribo-

somal protein, has been shown to be functionally equivalent to the X homologue *RPS4X* (Watanabe et al. 1993). Several of the other Y homologues are believed to be nonfunctional genes on the basis of truncated reading frames, and some of the genes that escape lack Y homologues altogether (see review in Disteche 1995). This suggests that, for such genes, dosage differences between the sexes either is not important or may be associated with a sex-specific or -limited function. One peculiar case is that of the synaptobrevin gene, which is located in the long-arm pseudoautosomal region of the human sex chromosomes. Instead of escaping X inactivation, like all pseudoautosomal genes examined so far, *SYBL1* is dosage compensated by inactivation both on the inactive X chromosome and on the Y chromosome (D'Esposito et al. 1996). Many of the genes that escape X inactivation but do not have a functionally equivalent Y homologue appear to be remnants of events that fashioned sex-chromosome evolution. Such events include loss of functional genes from the Y chromosome, acquisition of novel male-specific functions encoded by the Y chromosome, and rearrangements between autosomes and sex chromosomes (see review in Graves 1995; Ellis 1996).

The findings reported by Brown et al. in this issue of the *Journal* provide new opportunities to examine the question of stability of X inactivation in cell lines and in tissues. The identification of heterogeneity in cell lines, regardless of whether it occurs in vivo, will allow meaningful comparisons of the molecular characteristics of X-linked genes when they are inactivated or when they escape from inactivation. If confirmation of the heterogeneity of expression of X-linked genes found in hybrid cells is obtained in vivo, new ways of evaluating the phenotypic consequences of X-linked mutations will need to be derived. A final point emphasized by the findings of Brown et al. is that the identification of new genes that escape X inactivation provides new candidates for understanding the pathogenesis of Turner syndrome.

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