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X Chromosome Inactivation

No longer 'all-or-none'

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Exciting new data indicate that X-chromosome inactivation (XCI) is very far from the 'all-or-none' phenomenon that we thought it was when it was first discovered.

When XCI was first described, it was thought that one X-chromosome in somatic cells of female mammals was fully actively transcribed (Xa) and the other was completely inactive (Xi). This was seen as a dosage compensation mechanism, that is, a mechanism that ensured that XX females and XY males would have equal dosages of X-linked gene products.

Very quickly, however, it was suggested that X-linked genes with homologues on the Y-chromosome would escape inactivation as they would not require dosage compensation. While the suggestion that genes with Y-chromosome homologues would escape from inactivation was found to be true, other genes without Y-chromosome homologues were also found to escape XCI.[1,2] Thus the phenomenon was no longer all-or-none, in the sense of a whole chromosome being inactive or active. Nonetheless, the all-or-none concept of XCI persisted in that individual genes were thought either to escape fully or to undergo complete inactivation.

An exciting outcome of the human gene-sequencing project is that it has enabled much more detailed studies of the X-chromosome and XCI. A total of 1098 genes have now been identified on the human X-chromosome,[3] and Laura Carrel and Hunt Willard, in a recent *Nature* paper,[4] have been able to study escape from XCI in all of these genes that are expressed in cultured skin fibroblasts (over 600).

A striking and exciting feature of their results was that XCI was not all-or-none for every gene. About 20% of genes were inactivated in some but not all samples, and thus were expressed in either one or two doses in different samples. A further 15% escaped XCI completely, and so were expressed in two doses, and only 65% were fully silenced, and were thus expressed in the expected one dose only.

If these results with cultured cells reflect the situation *in vivo*, then Carrel and Willard's findings have important implications for clinical genetics. Genes without Y-chromosome homologues that escape XCI will have unequal dosages of their gene products in males and females. This could underlie some of the phenotypic differences between normal males and females. Genes with variable escape from XCI are also likely to underlie previously unexplained variation among females, either normal females or those heterozygous for X-linked disease genes. In the latter case, in addition to variation in the percent of cells having the mutant X-chromosome inactive, there will also be variation in the proportion of those cells in which the mutant gene is silenced.

These new results also provide increased understanding of the phenotypic abnormalities in individuals with X-chromosome anomalies, particularly those who are X0 or have partial deletions of the X-chromosome. For the escaping genes, the expression of two gene doses from the two X-chromosomes is normal. Females with deletions or who are X0 will have a deficit of the gene products of these genes in the deleted region. Escaping genes are not evenly distributed along the X-chromosome, but tend to be clustered,[4] notably in the distal region of Xp, and also in some other spots in Xp and in Xq. The concentration of escaping genes in Xp is consistent with the more severe effect of deletions of Xp than of Xq, since there are more genes in Xp for which expression of two doses is the normal state.

It will be fascinating to see how generally applicable these results in cultured cells are to cells *in vivo*. In addition to skin fibroblasts, the authors studied rodent–human somatic cell hybrids, in which XCI

is known to be somewhat less stable than normal, in that experimental treatments can reactivate some X-linked genes. In skin fibroblasts, on the other hand, XCI is typically highly stable. However, in marsupials, in contrast to eutherian mammals, XCI is much less stable. In some species, genes on the Xi are silenced in body tissues but undergo reactivation in skin fibroblasts.[5] Thus, it seems possible that in eutherians also, such as the human, although the majority of genes remain stably inactivated in skin fibroblasts, some may undergo reactivation while remaining silent *in vivo*.

If variable escape from XCI does indeed occur *in vivo*, it would be interesting to know whether specific genes behave the same in all cells of an individual female. It seems probably not. In the embryo, XCI is initiated independently in each cell present at that time. It is a complex process with several steps. There is first initiation of silencing, in which the X-chromosome is coated with noncoding RNA of the *XIST* gene, followed by a process of stabilization, involving histone modifications and methylation of CpG islands. Thus, particular steps could fail or not in individual cells.

In addition to its clinical interest, Carrel and Willard's work is also exciting for those studying the mechanism of XCI. Comparison of genes that escape with those that fully undergo XCI may reveal critical differences. Although the key gene *XIST* is necessary and sufficient for initiating silencing, the process of stabilization of XCI is independent of *XIST* and apparently involves several steps and genes.[6] Escape from XCI could result from resistance to the original signal or be due to failure of the stabilization process so that the gene is reactivated. We already know that in the mouse reactivation can occur, either of X-linked genes or of autosomal genes translocated to the X-chromosome.

Differences in CpG island methylation, or LINE1 DNA repeats, might also be involved in escape from inactivation. The X-chromosome is particularly rich in LINE1s, which have been suggested to take part in promoting XCI, and the new work[3,4] shows that LINE1s are not enriched in regions with many escaping genes, but the significance of this is not yet clear. Carrel and Willard also found that some escaping genes had less activity when on the Xi than when on the Xa. Thus, there are now a variety of completely silenced, partially escaping, and fully escaping genes whose features can be studied in attempts to reveal vital factors in the mechanism of XCI. These results have opened new avenues of research both on the variability of XCI in human females and on the mechanism of X-inactivation ■

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