INVITED EDITORIAL
XIST Expression and X-Chromosome Inactivation in Human Preimplantation Embryos

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The mosaic nature of females heterozygous for X-linked genes was one of the observations that originally contributed to Lyon's (1961) hypothesis of X-chromosome inactivation. This mosaicism results from the random silencing of one of the X chromosomes in somatic cells and is of considerable clinical importance as an influence on the severity of X-linked diseases in females (Willard 1995). X-chromosome inactivation, however, is not always random and can also be subject to imprinting. In marsupials it is always the paternal X chromosome that is inactivated (Cooper et al. 1971), and this same paternal bias is observed in rodents—but only in extraembryonic tissues (Takagi and Sasaki 1975; West et al. 1977). There is also evidence suggesting a parental bias in human placental trophoblast cells (discussed further below).

Although X-chromosome inactivation has been known and studied for many years, much remains to be discovered about the specific events involved, particularly those occurring during early development. Even determining the exact time of X-chromosome inactivation has proved difficult, since inactivation does not appear to occur at the same time in all tissues (Tan et al. 1993). In addition, inactivation of the entire X is not completed within a single cell generation, and the characteristics associated with inactivation (i.e., methylation, late replication timing, altered histone acetylation, and heterochromatinization) do not occur simultaneously (Gartler and Riggs 1983). Studies of artificial chimeras, combined with inferences of precursor-cell population size that are based on the frequency of X skewing within and between tissues have led to the conclusions that (1) commitment of the X chromosome to be inactivated has not yet occurred in the inner cell mass of the blastocyst, (2) $\geqslant$10–20 embryonic precursor cells must be present at the time that an X is designated to be inactivated, and (3) inactivation occurs prior to the differentiation of embryonic tissues. Therefore X-chromosome inactivation is assumed to be initiated in the embryonic ectoderm shortly after blastocyst implantation but takes several days to complete (Gardner and Lyon 1971).

A molecular clue to the process of X-chromosome inactivation was uncovered with the identification of the human *XIST* gene—a functional RNA expressed only from the inactive X chromosome (Brown et al. 1991). Knockout experiments of the mouse homologue, *Xist,* have shown that the Xist RNA is required for X-chromosome inactivation in females (Penny et al. 1996; Marahrens et al. 1997), and transgenic experiments have shown that the *Xist* gene with only 15 kb of flanking sequences is sufficient to cause inactivation (Herzing et al. 1997). *Xist* expression has been detected from 8-cell-stage embryos of mice, and this expression is imprinted, correlating with the nonrandom inactivation seen in murine extraembryonic tissues. Exclusive expression of the paternal allele is observed until shortly before gastrulation—the same time at which the first random X inactivation is seen (Marahrens et al. 1997). Female mice inheriting deletions of the *Xist* allele from their fathers are not viable, presumably because of the inability to dosage compensate in the extraembryonic tissues (Marahrens et al. 1997). Similarly, it has been hypothesized that the early developmental failure of mice carrying an extra maternally derived X chromosome (but not a paternally derived one) is due to the inability to inactivate the extra X chromosome (Takagi 1991).

In this issue of the *Journal,* Daniels et al. (1997) examine the expression of the human *XIST* gene in preimplantation embryos and show that *XIST* expression is occasionally detectable in the zygote and is more consistently seen in 4–8-cell embryos. Although this expression may be slightly earlier than has been observed in mice, what is curious in the present study is that expression is detected in both male and female early embryos, suggesting that the maternal X inherited by males is capable of expressing *XIST*. This is in strong contrast with the imprinted paternal expression of *Xist* in mice, which has been suggested to predispose the paternal X

Received May 7, 1997; accepted for publication May 13, 1997.

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This article represents the opinion of the authors and has not been peer reviewed.

0002-9297/97/6101-0003$02.00

to undergo inactivation—resulting in nonrandom inactivation of the extraembryonic tissues, which undergo inactivation before the imprint is erased (Kay et al. 1993). How, then, can the maternal *XIST* expression in humans be reconciled with the issue of nonrandom X-chromosome inactivation in human extraembryonic tissues? We address this question in terms of a number of component questions and, in figure 1, have summarized many of the points.

Do Humans Have Nonrandom X Inactivation in Their Extraembryonic Tissues?

In contrast to the situation in rodents, the evidence for nonrandom inactivation in human extraembryonic tissues has not been definitive. Early studies using isozyme analysis of the glucose-6-phosphate dehydrogenase (G6PD) enzyme were confounded by maternal contamination, isozyme-detection bias, and detection of expression from both X's (Migeon and Do 1979; Migeon et al. 1985). Nonetheless, most studies indicate

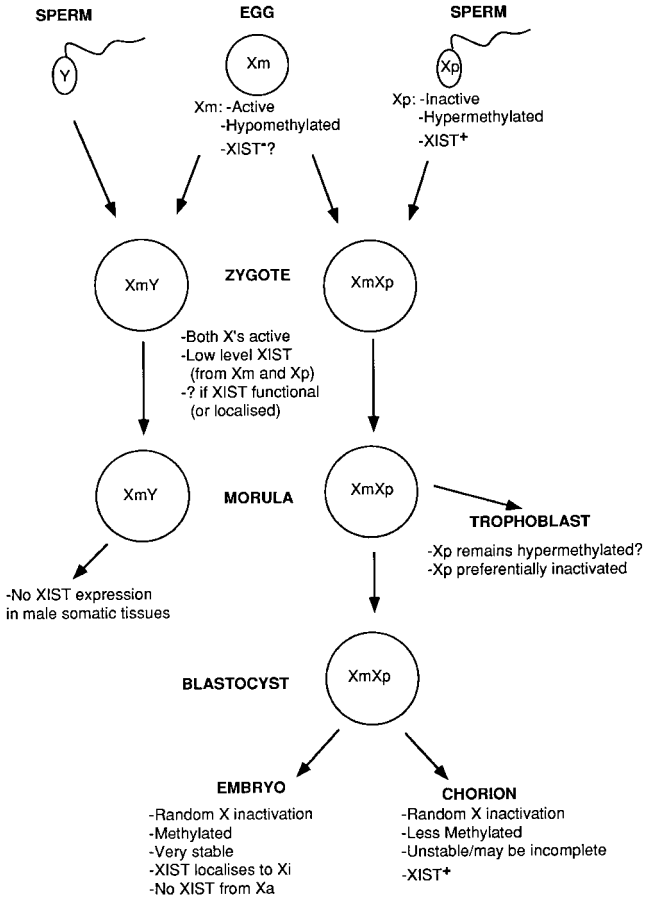

Figure 1 *XIST* expression and X chromosome inactivation in early human embryos. Xm = maternal X chromosome; Xp = paternal X chromosome; Xi = inactive X chromosome; and Xa = active X chromosome.

that human amnion, chorion, and whole-chorionic villi (i.e., chorionic mesoderm and trophoblast) do not show exclusive inactivation with regard to parental origin, although a bias was detected in some studies. However, preferential inactivation of the paternal allele in trophoblast has been detected in the two studies that isolated this cell type from chorionic villi. In a study of cytotrophoblast cells isolated from term placenta, Harrison (1989) carefully controlled for confounding factors and showed preferential maternal expression of G6PD in 12 of 13 samples, with exclusive maternal activity in 5 samples. Recently, methylation analysis of the polymorphic androgen receptor gene (*AR*) was used to show preferential paternal inactivation in two uncultured trophoblast samples digested from 10–12-wk chorionic villi (Goto et al. 1997). This latter study relied on methylation, which is known to be more variable in chorionic villi than in somatic tissue (e.g., see Luo et al. 1993). Additionally, because of the clonal nature of placental tissue (Harrison et al. 1993), very small samples will likely show extreme skewing in X inactivation by chance alone, and therefore it is difficult to evaluate whether the observed skewing in human trophoblast is due either to exclusive inactivation of the paternal copy or simply to a tendency in this direction. It is also unlikely—but theoretically possible—that *AR* methylation or *G6PD* expression in trophoblast is imprinted independently of the primary state of X inactivation.

What Is the Significance of Low Levels of *XIST* Expression Prior to X Inactivation?

Given the sensitivity required to measure expression from a limited number of cells, it is difficult to judge the significance of the *XIST* expression detected by Daniels et al. (1997). A low level of maternal *XIST* expression in males may be due simply to "leakiness" of the imprint, and there may still exist a parent-of-origin dependent–expression difference between the two chromosomes. Alternately, the expression of *XIST* from both maternal and paternal X chromosomes in early human development may be comparable to the expression observed in murine embryonic stem (ES) cells. In ES cells, low-level expression is observed from both the male and female X prior to differentiation (Panning and Jaenisch 1996). This RNA does not localize across the inactive X in the way that it does in somatic tissues, and, on differentiation, expression is lost in males, whereas in females this expression is lost from one X chromosome and *Xist* expressed from the other X paints the chromosome (Panning and Jaenisch 1996). Since the parental imprint resulting in nonrandom X-chromosome inactivation is apparently lost in ES cells (Sado et al. 1996), it is not known whether the low-level expression reflects a situation that is relaxed relative to that occurring in

embryos. It has, however, been shown that the expression of *Xist* in murine preimplantation development is very low—at least an order of magnitude below that of somatic cells (Latham and Rambhatla 1995). The level of *Xist* detected during mouse spermatogenesis is also low (Kay et al. 1993), and it has recently been shown that mice with deletions of *Xist* are fertile, suggesting that *Xist* expression is not required for spermatogenesis (however, inactivation during spermatogenesis has not been studied in these mice) (Marahrens et al. 1997). Therefore, low levels of *XIST* may not be sufficient for X inactivation. However, the process of inactivation seems to involve both silencing of the low level of expression from the active X and increasing expression of *XIST* from the inactive X, which is then localized to the inactive X. Thus, the choice of chromosome to be inactivated may well be influenced by an imprint that either limits the expression of *XIST* from the maternal X or predisposes to expression from the paternal X chromosome.

Could There Be Heterogeneity in the Imprinting of *XIST?*

The early expression of *XIST*—and the subsequent nonrandom inactivation of the X chromosome—may be subject to imprinting, but this may not be an all-or-none phenomenon. There is, in fact, indirect evidence to suggest that, although the paternal X may be preferentially inactivated in some human tissues, it is not a requirement for normal human development. In humans there is no difference in the phenotype of X aneuploidies (XO or XX, XXY) based on parental origin (unlike the situation in the mouse, as mentioned above). Additionally, paternal uniparental disomy of the X has been reported without major phenotypic effects attributable to imprinting (Schinzel et al. 1993). Furthermore, families have been reported that appear to have preferential activation of the paternal X chromosome (although inactivation status in placental tissues has not been examined) (Naumova et al. 1996). Heterogeneity in imprinting status could occur within an individual if some cells retain the imprint while others do not, or it could be polymorphic between individuals, in a situation analogous to the biallelic expression detected for some imprinted genes in some placentas (Jinno et al. 1995).

Is Imprinted *XIST* Expression Necessary for Nonrandom Inactivation in Extraembryonic Tissues?

Since *XIST* expression has not yet been examined in trophoblasts, a preferential paternal inactivation of an X chromosome in this tissue may not be associated with imprinting of *XIST* at all. Instead, a bias toward inactivation of the paternal chromosome may be due simply to the fact that the paternal X is transmitted from the sperm to the fertilized zygote in a highly methylated and inactive state. This may cause the paternal X to be more receptive to the inactivation signal than is the hypomethylated maternal X chromosome.

Although *XIST* expression is required for X inactivation, it is likely only one step of a multistep process. Additional factors are presumably required to transform the chromosome into an inactive state, and these factors may differ between extraembryonic and embryonic tissues. Differences in this process are evidenced by the observation that DNA from the inactive X chromosome in primitive ectoderm derivatives is ineffective in cellular transformation whereas that from primitive endoderm derived can be effective (Kratzer et al. 1983), likely reflecting hypomethylation of these tissues. Additionally, X inactivation in chorionic villi is completely reversible (Luo et al. 1995).

Just as we cannot assume that the process of X inactivation in early extraembryonic tissues is truly the same as that in the embryo proper, we need also to be careful in assuming that gene processes occurring in the mouse can always be extrapolated to the human situation. Understanding the timing and role of imprinting in X inactivation specifically for humans is clearly important not only for understanding this fundamental genetic process but for predicting the clinical consequences of genetic and chromosomal abnormalities involving the X chromosome.

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