Is *Tsix* repression of *Xist* specific to mouse?

Because X-chromosome inactivation is a uniquely mammalian method of dosage compensation and much of the mammalian X chromosome has been conserved[1], the mechanisms underlying the single active X are probably the same for most mammals. But some features differ, even among tissues of an individual. These differences have to do with whether X inactivation is imprinted (the maternal X is always active) or random (either X can be active) and the stability of the inactive state. Underlying such variations are species differences in the physical map of the X inactivation center, temporal differences in the onset of developmental events and the role of tinkering in evolution of biological processes[2,3]. Such changes usually eliminate or add elements that modify, but do not interfere with, the basic blueprint. Because X dosage compensation is an essential developmental program for mammalian cells, clearly these variations do not meddle with the basic mechanisms.

The gene *Tsix* represents an important species difference, perhaps related to the fact that X inactivation is imprinted in mouse but not human placenta[4]. In the mouse, *Tsix* transcripts are antisense to mouse *Xist* and inhibit expression of the maternal *Xist* allele in placental cells[5] and of the future active X in embryonic stem cells[6,7]. By repressing the accumulation of *Xist* transcripts, *Tsix* blocks the cascade of events that lead to transcriptional inactivation. On the sole basis of observations in mice, *Tsix* has been proposed to have an essential role in protecting the future active X from inactivation—not only when the X is maternal, but also when randomly chosen[8,9].

But the human version of the gene does not share this function[10,11]. Human *TSIX* is antisense to *XIST* but carries a deletion of the CpG island, which was shown by Lee and colleagues to be essential for function of *Tsix*[7,9]. There is evidence that CpG islands, like the one missing from *TSIX*, are needed for imprinting[12,13], consistent with lack of imprinted X inactivation in human placenta. Most important, *TSIX* transcripts are ineffectual; they do not repress *XIST* in *cis* and, in fact, are co-expressed with *XIST* from the inactive X throughout human embryonic development[11]. Despite being homozygous with respect to this *TSIX* mutation, human females undergo random X inactivation. Therefore, *TSIX* can not be essential for this function in our species. In addition, X inactivation in bovine placenta is imprinted[14], but evolutionary changes in the region 3′ to the bovine *Xist* gene[15] suggest that bovine *Tsix* may also be defective. Conceivably, *Tsix* regulation of *Xist* is an exclusive feature of X inactivation in the mouse. After all, the evolution of laboratory mice has been subject not only to chance events but also to considerable artificial selection.

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—In reply

Migeon argues that the regulation of X-chromosome inactivation differs considerably between mice and humans. Specifically, she suggests that antisense regulation of *Xist* by *Tsix* occurs only in mice, where the antisense *Tsix* blocks *Xist* expression during both imprinted and random X inactivation[1–7]. Migeon and colleagues have shown that the human X-inactivation center also expresses a transcript antisense to *XIST*[8], but conclude that human *TSIX* cannot be functional for two reasons: (i) *TSIX* truncates within *XIST* intron 4 and (ii) *TSIX* is sometimes co-expressed with *XIST* in differentiated cells[9]. It is thus argued that human females are *TSIX*-deficient and that *TSIX* cannot be a regulator of human X inactivation.

But are these conclusions premature? Human females are not *TSIX*-deficient, as an antisense RNA is observed by both RNA fluorescence *in situ* hybridization and RT–PCR[8]. That *TSIX* has only partial overlap with *XIST* does not *a priori* exclude a repressive role. In prokaryotes, antisense genes can show <100 bp of complementarity to sense targets[10]. Thus, it could be argued that *Tsix* RNA duplexes with and titrates out *Xist* RNA and does so with only partial complementarity. Alternatively, if antisense transcriptional activity provides the repressive force, transcription through the 3′ half of *XIST* may be all that is necessary, or *Tsix* may work through DNA elements embedded in the antisense locus[4,11].

Recent analysis of mouse *Tsix* RNA structure and abundance has implications for the interpretation of the human studies[12]. *Tsix* is spliced to eliminate all but a 1.9-kb domain complementary to the 5′ end of *Xist* RNA[6,12], *Xist*'s silencing domain[13]. Therefore, if human *TSIX* were similarly spliced, the detection of intronic regions would be relatively difficult. Furthermore, the analysis shows a gradient of *Tsix* expression, with 10 times more RNA made at its 5′ end than the 3′ terminus[12], suggesting that much of *Tsix* transcription ends before it crosses all of *Xist*. Human *TSIX* may have a similar gradient. Lack of detectable distal transcription may, in fact, reflect fewer transcripts rather than actual termination of transcription. A

quantitative approach is necessary to resolve this issue.

Finally, there is the concern that human *TSIX* has only been characterized in cell types that may not be physiologically appropriate. For example, because early truncation of *TSIX* was observed on a transgene in mouse embryonic stem cells[8], one caveat is that transcriptional regulation of the human transgene may not be fully recapitulated in mouse cells. Furthermore, for practical reasons, co-expression of *XIST* and *TSIX* was observed in primordial germ cells and fetal fibroblasts[9] rather than at the peri-implantation stage when X inactivation actually takes place.

Without further study, it is too soon to say whether *Tsix*-mediated regulation is shared by humans and mice. So far, the primary underpinnings of X inactivation seem identical in the two organisms. Given that the human has been so well modeled by the mouse in general, it seems reasonable to predict that X inactivation will be similarly regulated in the two.

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