DEVELOPMENT

Programming the X Chromosome

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A legacy of sex in mammals is the imbalance in the number of X chromosomes—females are XX and males XY. To overcome this imbalance, there is permanent inactivation of one of the two X chromosomes in every cell in females. The quest to unravel the precise mechanism of X inactivation continues, as three recent studies make clear (*1*–*3*). All three studies broadly agree that it is the paternal X chromosome (X^p) that is initially "imprinted" to undergo inactivation, and indeed, it is X^p that is inactive in placental tissues. However, within the embryo itself, either X^p or X^m is randomly selected for inactivation, resulting in a mosaic pattern that persists in adult cells. On pages 644 and 666 of this issue, Okamoto *et al*. (*1*) and Mak *et al*. (*2*) now reveal how this is achieved. They show that the paternal imprint is erased from X^p at a later stage from a group of pluripotent cells that subsequently give rise to the fetus. This erasure is then followed by random X inactivation. These studies provide important insights concerning not only X inactivation, but also aspects of early mammalian development and pluripotency.

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The prevailing view has been that X inactivation occurs in the blastocyst, which consists of the outer trophectoderm cells and the inner cell mass (ICM) at embryonic day 3.5. The developing ICM itself eventually contains primitive endoderm cells and an inner core of pluripotent epiblast cells, which are the precursors of both the fetus and pluripotent embryonic stem (ES) cells (see the figure). The preferential inactivation of X^p is first detected in differentiating trophectoderm and primitive endoderm cells, the first definitive tissues to form during development. In the embryo proper, there is random inactivation of X^m or X^p that commences at the onset of gastrulation around embryonic day 6.5.

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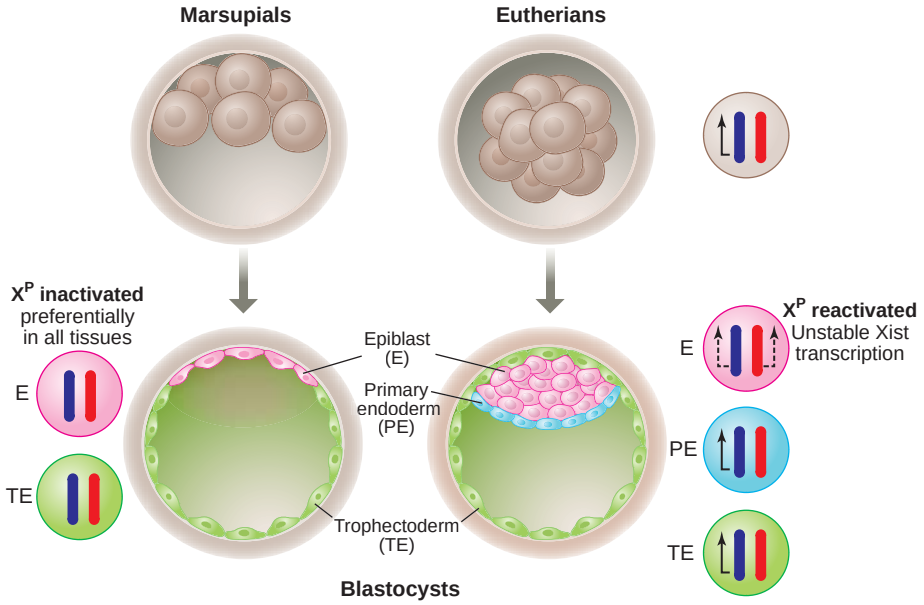
Recent studies have started to provide detailed knowledge of some of the early steps that precede the overt manifestation of X inactivation. Using slightly different approaches, Huynh and Lee (*3*) report in a recent issue of *Nature* that the paternal X is already silent at fertilization, albeit not entirely, and this is evident as early as the two-cell stage. In contrast, Okamoto and colleagues show that X^p silencing does not become evident until the four-cell stage (*1*). These differences notwithstanding, both groups assign a key role in the initiation of X inactivation to a long noncoding RNA molecule called Xist (X-inactivation specific transcript). Starting very early during development, possibly at the two- or four-cell stage, Xist coats the X chromosome that is destined for inactivation (*1*, *3*). This event, directly or indirectly, is the signal for the recruitment of Ezh2/Eed, a protein complex known to be associated with gene silencing (*4*–*7*). This complex has enzymatic activity and is able to modify proteins called histones that are intimately associated with the DNA of chromosomes. Their principal modification is to add methyl groups (methylation) to lysine 27 and possibly to lysine 9 of histone H3 (H3K27/K9). This accumulation of Eed/Ezh2 and associated methylation of H3K27/K9 is a relatively early mark of X inactivation. There are other forms of histone modification known to be associated with the initiation of X inactivation, including methylation of H3K9 that is detected slightly later at the 32-cell stage (*1*). The key point, however, is that Xist and Eed/Ezh2 together with the methylation of H3K27 are involved only in the initiation of X inactivation, because subsequent maintenance of the inactive X is independent of Xist. In the long term, the early modifications of histones are followed by more enduring modifications of the DNA itself, namely, methylation of critical cytosine residues on the X chromosome.



A multilayered advantage for eutherian mammals. Early development and X inactivation in marsupials and eutherian mammals. **(Left)** Both the epiblast (E) and trophectoderm (TE) cells of the single-layered marsupial blastocyst show preferential inactivation of the paternal X chromosome (X^p; dark blue), but the mechanism for the initiation of X inactivation in marsupials is unknown. **(Right)** By contrast, in the multilayered eutherian mouse blastocyst, only the trophectoderm and primary endoderm (PE) show preferential paternal X inactivation, which is due to stable Xist transcription (black arrow) from X^p. The pluripotent epiblast cells within the inner cell mass represent a unique "reprogramming" niche for the reactivation of the imprinted paternal X chromosome represented by two unstable Xist transcripts (broken black arrows). During subsequent development of epiblast cells, there is random inactivation of either the paternal or maternal (red) X chromosome in the fetus.

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There are at least two formal possibilities to account for the observed differences between the random inactivation of X in the fetus and the preferential inactivation of X^p in the placenta. First, certain cells within the early morula (the stage at which the embryo consists of a cluster of only a few cells) may escape imprinted X^p inactivation; these cells may then contribute to the ICM, a possibility suggested by Huynh and Lee (*3*). However, it is also known that asymmetric division of the polarized eight-cell blastomeres is responsible for creating the inner cells destined for the ICM (*8*), which implies that cells in the ICM may not be set aside early in development. The work of both Okamoto *et al*. (*1*) and Mak *et al*. (*2*) favors the latter possibility. They show that the assembly of the Eed/Ezh2 complex and the associated histone modifications on X^p are not uniquely confined to trophectoderm and primitive endoderm cells, but surprisingly appear in most cells at the late morula stage. It is noteworthy that this status persists in most if not all cells of early blastocysts, including the ICM. This is then followed by a progressive reversal or erasure of events associated with X^p inactivation within the ICM. This process occurs exclusively in the inner core of pluripotent epiblast cells and is not observed in the primitive endoderm cells of the ICM, which clearly implies that only the epiblast cells have this unique property.

Recently it was shown that the inner cells of the late morula exhibit expression of the *nanog* gene, which is subsequently confined to the epiblast cells and is not observed in the primary endoderm cells (*9, 10*). The *nanog* gene is critical for pluripotency of epiblast and ES cells, and its expression is down-regulated as cells differentiate. The reversal of X^p-associated changes may therefore require development of the epiblast cells. However, the precise way in which this process is triggered and its underlying mechanism remain unknown. Nonetheless, the result is subsequent random inactivation of X^p or X^m when all the steps involving Xist, Eed/Ezh2, and histone modifications are recapitulated in differentiating epiblast or ES cells.

For comparison, it is important to note that marsupials exhibit preferential inactivation of X^p in all tissues, although this silencing of the X is not as robust as in eutherian mammals (*11*). There is also a striking difference in early development between eutherian mammals and marsupials that could provide an explanation for the differences between them (see the figure). Mouse blastocysts are multilayered with the outer trophectoderm cells surrounding the ICM, which is located at one end of the blastocyst. In contrast, marsupial blastocysts have only a single layer with epiblast and trophectoderm cells located at opposite ends of the blastocyst (see the figure). It is possible that this fundamental difference in their development provides a unique niche within the ICM of mouse blastocysts for development of pluripotent epiblast cells, which then acquire the remarkable property of erasure of the paternal imprint from X^p. Because X reactivation might be an important component of reprogramming of the genome, it is possible that some of the factors implicated in the establishment and maintenance of pluripotent epiblast cells may be involved in this process. These key factors must also be present in ES cells because the inactive X chromosome is reactivated in the nuclei of hybrids composed of ES cells and somatic cells (*12*). By following the fate of X^p in early embryos, it may be possible to gain further insight into not only X inactivation but also aspects of early mouse development, pluripotency, and reprogramming of the genome.

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CHEMISTRY

Throwing Tetrahedral Dice

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Surfaces and interfaces of liquid water and ice play a crucial role in a plethora of different processes. Understanding even bulk water, however, has posed scientific challenges for decades, in addition to stimulating the imagination of metaphysicists. Still, a consistent picture of pure bulk water at ambient conditions is now emerging both experimentally (*1*, *2*) and in ab initio simulations (*3*–*6*). Given this favorable situation, researchers can now turn to the study of aqueous solutions, surfaces, and interfaces. On page 658 of this issue, Kuo and Mundy (*7*) report on an exceedingly large-scale ab initio simulation (*8*, *9*) of the surface of liquid water. In addition to being a significant leap forward in size, this proof-of-principle study offers important molecular insights, especially in relation to recent experimental findings as to how this hydrogen-bonded surface is terminated.

H_2O is a network-forming molecule that can be thought of as having a tetrahedral shape: Covalent OH bonds point into two corners of an imaginary tetrahedron, and the two lone electron pairs can be associated with the remaining corners (see the figure). Hydrogen bonds between two water molecules can be made when a lone pair from one molecule, acting as the acceptor site (A), engages a proton from another, which is the hydrogen bond donor (D). Every water molecule can, ideally, accept and donate two hydrogen bonds, denoted as $(n_A; m_D)$, where $n = 2$ and $m = 2$. This property permits it to serve as the nucleus of beautiful three-dimensional networks, leading ultimately to an ever growing family, which currently numbers about ten ice phases, not to mention the intriguing liquid and amorphous states.

How does this feature of water extend to inhomogeneous surfaces and interfaces? Based on the above simplistic topological model, there are several ways in which an interfacial water molecule can remain hydrogen bonded: The tetrahedron can be attached either via a single corner, an edge, or one face to a plane. Furthermore, the A and D sites can be permuted among the different vertices, keeping in mind that there are at most two acceptor and donor sites available (that is, $n \leq 2$ and $m \leq 2$). The resulting seven possible interfacial connectivities of a water molecule are depicted schematically in the figure. The remaining two species, $(0_A; 0_D)$ and $(2_A; 2_D)$, shown above and below the plane, are involved in either zero or four hydrogen bonds and thus could be termed "vaporlike" and "bulklike" molecules, respectively.

Up to this point, the only possibilities explored have been those arising from the simple idea that water is geometrically a tetrahedron, somewhat like throwing tetrahedral dice. When it comes to deciding which of these interfacial species are actually preferred, surface-sensitive experiments (*10*) and simulations must be used.

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