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Do X Chromosomes Set Boundaries?

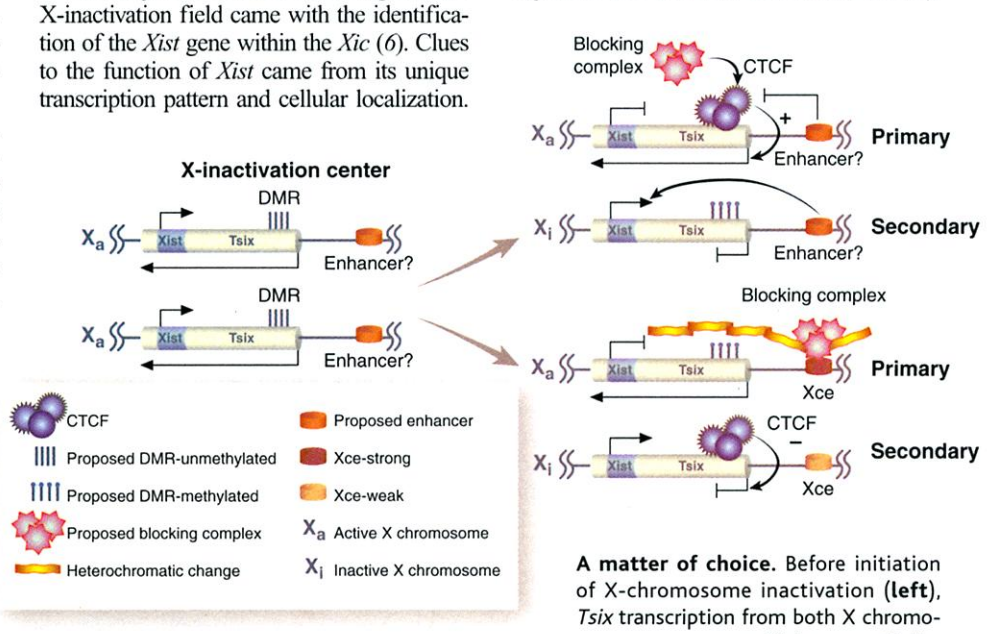
Ivona Percec and Marisa S. Bartolomei

Sexually dimorphic organisms employ the services of epigenetics—heritable changes in gene expression that are independent of DNA sequence—to balance genetic differences between the two sexes. A superb model of this relationship, X-chromosome inactivation, has evolved uniquely in mammals to ensure equal gene dosage between females, who have two X chromosomes, and males, who have only one X. This precise pathway results in the silencing of the majority of genes on one X chromosome early in female development. This outcome requires a female cell to undergo a highly orchestrated set of events when it differentiates. A cell must count the X chromosomes, choose one X to inactivate (usually in a random manner), initiate and propagate chromosome-wide silencing, and finally maintain this inactive state throughout subsequent cell divisions (*1*). Shortly after the discovery of X inactivation by Mary Lyon in 1961, geneticists hypothesized that cis-acting factors (acting on the same chromosome) encoded by the X must be important in this process. Likewise, trans-acting factors (acting on different chromosomes) encoded by chromosomes other than the X or Y were presumed to be equally important (*2*). Yet until recently, all known regulators of X inactivation were cis-acting elements residing on the X chromosome. The drought surrounding the identification of trans-acting factors has now ended. According to Chao *et al.* (*3*) on page 345 of this issue, the insulator and transcription regulator CTCF is a key trans-acting factor in the X-inactivation pathway.

Early studies on X inactivation demonstrated that a region of the X chromosome, designated the X-inactivation center (*Xic*), is required for silencing of adjacent sequences (*4*). As a result, a chromosomal fragment containing the *Xic* can become inactive, whereas one that does not, by default, must remain active. In addition to delineating the *Xic* as the principal cis-acting silencing center, early experiments uncovered a genetic element within the *Xic* that affects X-chromosome choice in the mouse (*5*). Alleles of this element, named the X controlling element (*Xce*), vary in strength such that a strong *Xce* allele is more likely to reside on an active X chromosome than a weak *Xce* allele. Surprisingly, *Xce* has escaped molecular identification.

The major molecular breakthrough for the X-inactivation field came with the identification of the *Xist* gene within the *Xic* (*6*). Clues to the function of *Xist* came from its unique transcription pattern and cellular localization. *Xist*, a gene that does not encode a protein, is transcribed from the inactive X chromosome (X_i) and is silent on the active X chromosome (X_a). It codes for a large untranslated RNA that coats the X_i. Genetic experiments have demonstrated that *Xist* is required for initiation and promulgation of silencing, and that it is involved in X-chromosome choice (*1*). These findings invoked a compelling molecular model of initiation and propagation events, with the *Xist* RNA acting as the major inactivating element. Despite this progress, molecular candidates directing the initial events of counting and selection remained elusive.

Studies of the antisense gene *Tsix*, the most recent addition to the cis-acting family of factors within the *Xic*, have begun to illuminate these early events (*7*). *Tsix* overlaps with *Xist*, but is transcribed from the antisense strand. Like *Xist*, *Tsix* codes for an untranslated RNA, yet contrary to *Xist*, *Tsix* is transcribed from the X_a. This pattern suggests that the two genes are coordinately regulated and that *Tsix* blocks *Xist* activity.



A matter of choice. Before initiation of X-chromosome inactivation (**left**), *Tsix* transcription from both X chromosomes suppresses *Xist* gene activity, preventing X-chromosome silencing. During X-chromosome choice, CTCF may bind to the future X_a as a primary event preventing *Xist* transcription (**top right**). In this scenario, suppression of *Xist* by CTCF could be achieved by direct activation of its repressor, *Tsix*, or by blocking access to putative enhancers located downstream. Alternatively, a blocking complex may bind to the future X_a as a primary event inducing heterochromatic changes within the *Xic*, including methylation and suppression of *Xist* (**bottom right**). In this scenario, CTCF binds to the future X_i as a secondary event and either directly represses *Tsix*, or blocks *Tsix*'s access to enhancers close to *Xist*. The enhancers have not yet been identified, and their location is speculative.

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Intriguingly, *DXPas34*, a region downstream from the *Tsix* promoter, is hypermethylated on strong *Xce* alleles (*8*). Recent experiments have shown that *Tsix* transcription from both X chromosomes before initiation of X inactivation suppresses *Xist*, thereby preventing X-chromosome silencing. As a result, *Tsix* may maintain the active state of the two X chromosomes before the onset of silencing (*1*). Genetic evidence suggests, however, that the function of *Tsix* extends beyond this simple model. *Tsix* also regulates X-chromosome choice, making it the third element within the *Xic*, along with *Xce* and *Xist*, postulated to act in this process (*1*).

Chao *et al.* (*3*) tackle this poorly understood stage of the inactivation pathway by searching for trans-acting molecules. Most models of X-chromosome choice invoke trans-acting blocking factors that protect the future X_a from silencing (*2*). The authors applied computational analysis to the *Tsix* promoter/*DXPas34* region and identified a mouse-specific cluster of binding sites for the ubiquitous chromatin insulator and transcription regulator CTCF. Insulators, first described in *Drosophila*, are defined operationally by their ability to protect genes against position effects and to prevent interactions between promoters and enhancers when positioned between them (*9*). In vertebrates, CTCF operates at the chicken β-globin gene locus (*10*) and at the imprinted *H19-Igf2* locus in the mouse (*11*). The current understanding of insulators is incomplete, however, and their mechanism of action remains obscure.

The investigators propose that CTCF and *Tsix* work together to direct X-chromosome choice. According to their model, CTCF binds first to one of the two X chromosomes, designating it as the future X_a and preventing *Xist* transcription. Allelic methylation differences within a proposed differentially methylated region (DMR) would enable discrimination of the X chromosomes and binding of CTCF to only one allele of the *Tsix*. Suppression of *Xist* by binding of the CTCF to the X_a could be achieved by direct activation of its repressor, *Tsix*, or by blocking access to putative enhancers located downstream (see the figure). The model proposed by Chao *et al.* provides an alluring solution to the long-standing dilemma of how a cell could discriminate between two seemingly equivalent X chromosomes. In an elegant portrayal of an epigenetic switch, it fulfills many of the requirements of X-chromosome choice. The model is consistent with the authors' previous observation that deletion of the CTCF array results in nonrandom inactivation of mutated X (*1*). Importantly, the study encourages the discovery of additional trans-acting factors, including those involved in the proposed blocking complex.

Nevertheless, questions remain about the specific function of CTCF in this setting, and the proposed enhancer and DMR have yet to be identified. Fortunately, many details of the epigenetic switch model are testable. For example, although Chao *et al.* demonstrate that the sites could bind CTCF in vitro and in vivo, it remains to be seen whether binding of CTCF is limited to one allele. If proven, how is monoallelic binding achieved in a developmentally specific and dosage-sensitive manner? How does the *Xce* effect on choice correlate with that of CTCF binding? Does hypermethylation of *DXPas34* affect CTCF binding? Does CTCF operate similarly in the human, where *Tsix* structure has been shown to vary from that of the mouse and where there is no known *Xce* effect? Could CTCF bind biallelically but invoke distinct allele-specific regulatory effects? One could envision an alternative model in which CTCF binds and functions secondarily after blocking complex–induced silencing of the *Xic* on the X_a (see the figure). Now that the gates to the garden of trans-acting factors have been thrown open, details of the epigenetic-switch model should rapidly follow.

The identification of CTCF as a trans-acting factor in the X-inactivation pathway provides not only significant insight into X-chromosome selection, but also raises awareness about the universality of epigenetic gene regulation. In the postgenomics era, genetic and epigenetic studies will once again become critical to understanding how exquisitely specific effects are achieved with global factors like CTCF. As additional facets of the pathway are revealed, X-chromosome inactivation will continue to provide an exceptional paradigm for gene regulation.

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PERSPECTIVES: CHEMISTRY

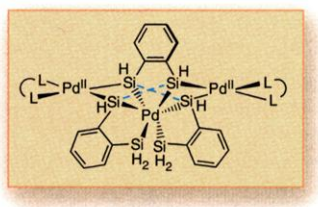
A New Oxidation State for Pd?

Robert H. Crabtree

The discovery of a new oxidation state of a transition metal element is rare. It is even more unexpected in the case of a long-studied metal like palladium (Pd). This precious metal of the platinum group forms exceptionally efficient catalysts (*1*) for a wide variety of organic reactions. Either as a Pd compound or as the finely divided element, these catalysts are widely used, for example, in the pharmaceutical industry.

Knowledge of the range of oxidation states accessible to an element is crucial in characterizing the mechanisms of catalytic reaction cycles. For a mechanism to be plausible, all postulated intermediates in such a cycle have to be assigned to a known metal oxidation (or valence) state.

In the late 1970s and early 1980s, only Pd(0) and Pd(II), with oxidation states 0 and +2, respectively, were believed to form in organic reaction cycles catalyzed by Pd. Pd(IV) was known in inorganic compounds such as PdO_2 or PdF_4, but these compounds are quite unlike the organometallic species, with Pd-C bonds, that are typically formed in catalytic cycles. Historically, the highest oxidation states had been seen with electronegative ligands, such as O and F (*2*). Organometallic compounds tended to have lower oxidation states and more electropositive ligands, such as C or Si.



The structure of the new Pd(VI) compound as determined by Chen *et al.* (*5*).

In the past 15 years, it has become fully accepted that organometallic compounds can contain Pd(IV) (*3*). Such compounds can be formed not just as unstable intermediates but as species stable enough for their structures to be determined by x-ray crystallography—the gold standard of chemical structure determination. But no definitive evidence for an organometallic Pd(VI) species has been presented (*4*).

On page 308 of this issue, Chen *et al.* (*5*) report the synthesis and x-ray crystallographic characterization of such a

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