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A tetrad of chromatin interactions for chromosome pairing in X inactivation

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Abstract

An unusual pairing of homologous X chromosomes occurs during X inactivation. A new study in mouse embryonic stem cells shows that telomeres and the telomeric RNA PAR-TERRA are responsible for additional pairwise interactions that guide Xic–Xic pairing.

Inactivation of one X chromosome in female mammals occurs early in development to balance expression of genes on the X chromosome with that in XY males^{1,2}. The X-inactivation center (Xic), a 15-kb region containing non-coding genes *Xite*, *Tsix* and *Xist*, drives this process (Fig. 1). Allelic choice randomly designates an X chromosome to be active (Xa) or silent (Xi). The X-inactive-specific transcript, *Xist*, coats the chosen Xi chromosome to initiate silencing, whereas the *Xist* antisense RNA, *Tsix*, which is positively regulated by *Xite*, antagonizes *Xist* on the chosen Xa chromosome to prevent silencing. A great deal of work has centered on the *Xist* RNA, including what proteins it recruits and how it silences genes on the chosen Xi. However, studies of X-chromosome inactivation (XCI) have increasingly used chromosome contact mapping³ (3C, 4C, 5C and Hi-C) to define the topology of the Xi and Xa and to probe how their differences relate to function^{4–7}.

A key step in successful XCI is the change to monoallelic expression of *Tsix* on the future Xi. How this occurs is still unclear, but one significant event that is required is transient pairing of the homologous X chromosomes at the Xic^{8,9}. Without pairing, allelic choice is disrupted. X-chromosome pairing is one of only a few examples of chromosome pairing that have been described outside meiosis^{10–13}. In this issue, Chu *et al.*¹⁴ bring us closer to understanding how pairing sequences get together. They find that telomeres on the X chromosomes pair with each other *in trans* and with their respective Xics *in cis*. TERRA RNA, transcribed from the subtelomeric pseudoautosomal region (PAR), designated PAR-TERRA, provides the ‘glue’ for these interactions. These new findings suggest that Xic pairing takes place within the embrace of a tetrad of interactions that help the Xics find each other.

COMPETING FINANCIAL INTERESTS

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Searching for new participants in X-chromosome pairing, the authors investigated telomeres, based on previous observations of their proximity early in embryonic stem (ES) cell differentiation¹⁵. The authors performed 3D DNA FISH to determine distances between Xic elements and telomeric PARs on X chromosomes in differentiating female mouse ES cells from mice. These cells faithfully undergo transient Xic pairing and XCI between days 1 and 4 of differentiation. The authors found that X chromosome PARs also undergo transient pairing during the same time window in which Xic–Xic pairing takes place. The Y chromosome also has a PAR, and the same interaction was observed between X and Y chromosome telomeres during differentiation of male ES cells, suggesting that transient PAR–PAR pairing is a common phenomenon for sex chromosomes during ES cell differentiation. These observations raised the possibility of a role for telomeric pairing in XCI.

Telomeres produce a mixture of long noncoding RNAs, termed TERRA, though much about the origin and localization of TERRA transcripts remains unclear. Chu *et al.*¹⁴ used RNA and DNA FISH and orthologous assays to show that TERRA and PAR RNAs accumulate at distal X-chromosome telomeres in female mouse ES cells undergoing differentiation, suggesting these elements are potential sites of TERRA transcription. To test this possibility, the authors performed TERRA-capture RNA-seq using a biotinylated probe to telomeric repeats. TERRA cDNA sequencing confirmed that most reads (>99%) overlapped with X and Y chromosome PARs, confirming that they are a major source of sex-specific TERRA RNA production.

To identify sites in the genome occupied by PAR-TERRA, the authors employed CHIRT-seq, a hybrid RNA-chromatin binding assay optimized for PAR-TERRA. Applying a wide range of controls and bioinformatic algorithms, they discovered considerable overlap in the binding patterns of TERRA and PAR in differentiating female mouse ES cells. The data support the idea that there are PAR-TERRA and TERRA telomeric transcripts, but PAR-TERRA transcripts predominate in ES cells. PAR-TERRA primarily binds to PARs but can also bind to nontelomeric repeats. The FISH and CHIRT results also support the possibility that PAR-TERRA may be a contiguous transcript comprising sequences from the PAR through the telomere, but definitive evidence for this idea is currently unavailable. Overall, these findings show that PAR-TERRA binds to chromatin *in cis* and *in trans*.

To examine whether PAR-TERRA plays a role in X-chromosome pairing, PAR-TERRA was depleted using locked nucleic acids directed against PAR or TERRA. Depleting TERRA reduced PAR and vice versa, consistent with the proposal that the two transcripts are linked. PAR-TERRA depletion caused disruption of PAR–PAR pairing in both female and male mouse ES cells. Moreover, ectopic overexpression of PAR-TERRA from an auto-somal BAC transgene established *de novo* contact between the site of overexpression and X-chromosome telomeres, supporting a direct role in sex chromosome telomere pairing.

Intriguingly, CHIRT revealed foci of PAR-TERRA binding within the Xic, specifically in mouse ES cells, further suggesting a role in pairing. Indeed, pairing is negatively affected by depletion of PAR-TERRA. The authors then considered whether Xic pairing might be initiated through long-range interaction between PAR and Xic that would constrain the two Xic in nuclear space, thereby facilitating homology searching and interaction. Imaging

showed reduced Xic–PAR distance during the same time frame as XCI in mouse ES cells. Applying an orthologous approach, the authors performed 4C to allow unbiased evaluation of interaction between Xic and other genomic loci. They found significant interaction between the PAR and Xic. Comparison of the 4C and CHIRT-seq results showed that the PAR and Xic are covered with PAR-TERRA, suggesting involvement of the transcript in PAR–Xic interaction. Indeed, depletion of PAR-TERRA caused disruption of the 4C interaction. In sum, these experiments show that PAR-TERRA is required for Xic–Xic, Xic–PAR and PAR–PAR pairing.

The observed interactions of Xic and PAR suggest the existence of a ‘tetrad’ of contacts involving these elements from two X chromosomes (Fig. 2). Strikingly, FISH showed that around 6% of differentiating mouse ES cell nuclei contain just such predicted structures. Depletion of PAR-TERRA caused significant reduction of the tetrads. While PAR–PAR pairing without Xic–Xic pairing was observed on individual alleles, the reverse was never the case. Taking into account that Xic–Xic pairing is essential for X inactivation, the authors proposed that PAR-TERRA may play an essential role in the process. Consistent with their prediction, depletion of PAR-TERRA precluded Xist cloud formation in differentiating female ESC and lead to continued biallelic expression of X-linked genes. These findings suggest that PAR-TERRA is required for homologous X-chromosome pairing and for successful XCI.

The X-chromosome tetrad of interactions is a satisfying leap in our understanding of how long-range interactions in chromosomes can form to influence the function of underlying sequences. Chu and colleagues show that two hooks are better than one. The observation of PAR–PAR pairing (and PAR–Xic pairing) without Xic–Xic pairing strongly supports the argument that PAR–PAR pairing precedes and probably aids Xic–Xic pairing. The role of PAR-TERRA RNA itself is intriguing. The PAR-TERRA transcript is seen throughout ES cells differentiation, yet Xic pairing is a brief, early event. There must be another factor or factors regulating the pairing event, perhaps a differentiation-specific factor. In addition, the continued transcription of PAR-TERRA during ES cell differentiation suggests it may have roles in other sex-linked functions that remain to be discovered. Also mysterious are PAR-TERRA’s other genomic binding sites—what is their function and do they participate in pairing? Beyond these questions, there is the fundamental issue of how the two X alleles find each other in the first place so that homologous and heterologous PAR pairing can occur. These and other questions will assure continued robust dissection of XCI in the future.

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Figure 1. Xic locus in mice. A simplified version of a portion of the X chromosome is depicted. Noncoding genes with known functions in XCI are indicated by colored rectangles. The region within the dotted rectangle is proposed to be involved directly in CTCF-protein-mediated pairing through multiple sites within *Xist*, *Tsix* and *Xite*¹⁶.

Figure 2.

A model depicting the contribution of multiple pairing interactions to Xic. PAR-TERRA binding to the PAR and Xic regions of X chromosomes has multiple potential outcomes (middle). PAR-TERRA can promote PAR-PAR pairing *in trans* as well as PAR-Xic pairing *in cis*. Either event can promote homology search and pairing by Xic, but Xic pairing does not occur without them. When Xic pairing does occur, it is in the context of a tetrad of interactions that contribute to close apposition of Xics and stabilization of their interaction. Chu and colleagues refer to this sequence as “constrained diffusion”¹⁴.