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CHROMOSOME BIOLOGY

OCT4 learns to count

> The pluripotency factor OCT4 regulates X chromosome inactivation…

The pluripotency factor OCT4 regulates X chromosome inactivation (XCI) by triggering X chromosome pairing and counting, report Jeannie Lee and colleagues in *Nature*. This study not only ascribes new functions to OCT4 that might be important for reprogramming somatic cells to pluripotent cells, but also identifies the first *trans*-acting factor that regulates X chromosome counting.

Female embryonic stem (ES) cells carry two active X chromosomes, but undergo transcriptional silencing of most genes on one of their X chromosomes when they differentiate. Previous studies have shown that blocking XCI disrupts ES cell differentiation and vice versa, suggesting that the two processes are tightly linked. Based on these findings, Lee and co-workers postulated that the same factors that are involved in stem cell pluripotency might also regulate XCI.

To test this hypothesis, they used bioinformatics to search for binding sites for pluripotency factors across the X inactivation centre (*Xic*), which is crucial for silencing the inactive X chromosome. They found candidate binding sites for OCT4 and SOX2 in *Tsix* and *Xite* — two regulatory non-coding RNA genes that repress *Xist* (a non-coding RNA that coats and silences the inactive X chromosome). Indeed, chromatin immunoprecipitation and electrophoretic mobility shift assays confirmed that OCT4 and SOX2 bind to *Xite*, and that OCT4 binds to *Tsix*, *in vivo* and *in vitro*.

The OCT4- and SOX2-binding sites in *Tsix* and *Xite* are located next to binding sites for CTCF and YY1, which regulate X chromosome pairing and determine which X chromosome will be inactivated during XCI. *In vivo* and *in vitro* experiments show that OCT4 interacts with CTCF and that SOX2 binds to YY1. Given that CTCF interacts with YY1, these results suggest that OCT4 is part of a multifactor complex on *Tsix*.

Next, the authors knocked down *Oct4*, *Sox2* and *Ctcf* in female ES cells using small interfering RNA (siRNA) and found that *Oct4* siRNA, but not *Sox2* and *Ctcf* siRNAs, reduced the expression of *Tsix* and *Xite*. So, OCT4 is required for the expression of *Tsix*.

X chromosome pairing is one of the earliest events of XCI and it occurs in the *Xic*. This process also regulates X chromosome counting and chromosome choice in XCI. Early knockdown of *Oct4* during ES cell differentiation causes defects in X chromosome pairing. Furthermore, RNA fluorescence *in situ* hybridization and quantitative reverse transcription PCR showed that *Xist* becomes biallelically expressed in a fraction of female ES cells that lack OCT4, which indicates a defect in X chromosome counting. Notably, these results also identify OCT4 as the first *trans*-acting factor for the counting process.

The authors propose that "the intrinsic developmental specificity of OCT4 — active in pluripotent cells and downregulated in differentiated cells — controls the timing of XCI by triggering pairing and counting" and links X chromosome reprogramming to ES cell differentiation and to de-differentiation of induced pluripotent stem cells.

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ORIGINAL RESEARCH PAPER Donohoe, M. E. *et al.* The pluripotency factor Oct4 interacts with Ctcf and also controls X-chromosome pairing and counting. *Nature* 17 Jun 2009 (doi:10.1038/nature08098)