Cell Research (2009) 19:917-919.
© 2009 IBCB, SIBS, CAS All rights reserved 1001-0602/09 $ 32.00
www.nature.com/cr

Oct4 to count 2

Anton Wutz[1]

[1]*Research Institute of Molecular Pathology, Dr. Bohr-Gasse 7, 1030 Vienna, Austria*
Cell Research (2009) **19**:917-919. doi: 10.1038/cr.2009.92; published online 3 August 2009

In mammals, dosage compensation between the sexes involves the transcriptional silencing of one of the two X chromosomes in female cells. Thereby, either the paternally or maternally inherited X chromosome becomes inactivated in a random manner. X inactivation is initiated in early embryogenesis. The X inactivation centre (*Xic*) regulates the initiation of X inactivation in a developmentally controlled process during early embryogenesis and contains elements that signal the presence of an X chromosome. The *Xic* also contains the non-coding *Xist* RNA, which localizes in *cis* over the X chromosome and triggers chromosome-wide gene silencing. The present view is that the mechanisms that control X inactivation converge at the regulation of *Xist*.

Xist expression has been studied in detail. *Xist* is repressed by the non-coding *Tsix* RNA that overlaps the *Xist* transcription unit in antisense orientation [1]. In female cells, *Tsix* is required to suppress *Xist* expression from the one X chromosome that will remain active. In male cells with only one X chromosome *Xist* remains repressed if *Tsix* is disrupted. This suggests that the presence of two X chromosomes predisposes female cells for *Xist* expression [2]. Before upregulation of *Xist* the *Xic* regions on the two X chromosomes pair providing a potential trigger for X inactivation [3, 4]. The current view is that the pairing event is responsible for establishing mutual exclusive outcomes on the active and the inactive X chromosome.

The molecular basis of pairing has been studied in embryonic stem (ES) cells. Female mouse ES cells possess two active X chromosomes, one of which becomes inactivated upon differentiation. Previous work by the Lee group has shown that the DNA binding protein CTCF is essential for *Xic* pairing, which is mediated through a region close to the *Tsix* promoter [5]. CTCF binds together with the transcription factor YY1 to multiple sites within the *Xic*, where it might contribute to *Tsix* regulation [6]. CTCF is expressed throughout ES cell differentiation and, thus, does not fully explain the developmental onset of X inactivation after the onset of differentiation. Yet, a recent study links *Xist* regulation and ES cell transcription factors [7].

In their present study, Donohoe and colleagues examine the function of the transcription factors Oct4 and Sox2, which show an expression pattern that is restricted to early ES cell differentiation (for review see [8]), in the developmental regulation of *Xist* [9]. Bioinformatic analysis reveals putative Oct4 and Sox2 binding sites within *Xist* and at the start site and enhancer of *Tsix* (Figure 1). Binding of the factors is confirmed by chromatin immunoprecipitation. Since Oct4 binding sites are close to CTCF binding sites the authors examine a potential interaction. They observe that CTCF interacts biochemically with Oct4, and Sox2 can bind YY1 suggesting the formation of a larger complex in ES cells. Importantly, mutation of Oct4 binding sites in the *Tsix* promoter reduces its transcriptional activity in a reporter assay. These observations implicate Oct4 in the regulation of *Tsix*. Oct4 and Sox2 are downregulated in differentiation of ES cells and, thus, could explain the loss of *Tsix* expression which in turn could enable of *Xist* expression and lead to the inactivation of the X chromosome. This interpretation is strengthened by the observation that Oct4 depletion using siRNAs leads to a reduction of *Tsix* expression and the expression of *Xist* from both X chromosomes in differentiated female ES cells. Thus, in this system Oct4 acts as a transcriptional activator of *Tsix* and a repressor of *Xist*.

The study further examines the role of Oct4 for *Xic* pairing. Depletion of Oct4 blocks *Xic* pairing indicating that Oct4 also is required for this process. The results by Donohoe *et al*. provide an important step forward for understanding the developmental regulation of X inactivation. The following model can be proposed: *Xic*-*Xic* pairing will predispose the cells for *Xist* expression. Yet, at the initiatial stage strong *Tsix* expression will prevent *Xist* upregulation. Upon differentiation Oct4 and Sox2 expression diminishes and leads to a shutdown of *Tsix* from one of the two X chromosomes allowing upregulation of *Xist* and X chromosome inactivation. In such a sense Oct4 could

Correspondence: Anton Wutz
Tel: +43-1-79730/3430; Fax: +43-1-7987153
E-mail: wutz@imp.univie.ac.at

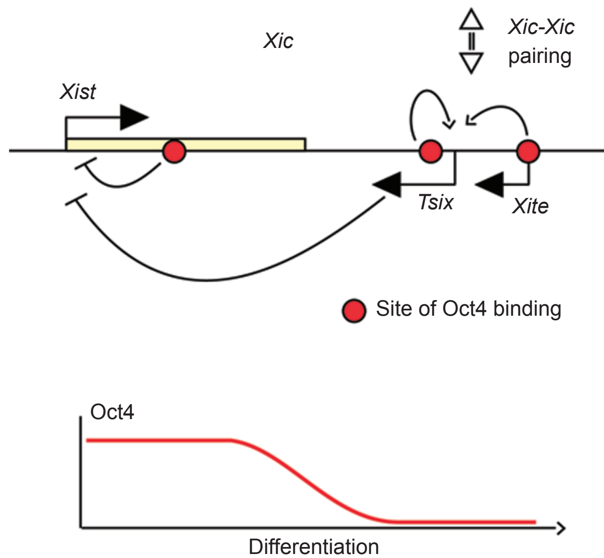

Figure 1 Summary of Oct4 binding in the *Xic*. A schematic representation of the mouse *Xic* region indicates the non coding *Xist* and *Tsix* RNAs. Oct4 binding to the *Tsix* promoter and its enhancer *Xite* activates *Tsix*, which in turn represses *Xist*. Down regulation of Oct4 upon differentiation leads to a reduction of *Tsix* transcription. This allows *Xist* upregulation from one X chromosome when *Xic-Xic* pairing triggers X inactivation.

provide a candidate for a blocking factor that has been proposed from theoretical considerations to explain how one X chromosome can be kept active. This is a significant step forward in understanding the regulation of X inactivation.

The results obtained by Donohoe and colleagues also prompt new questions and will spur further investigation. Among the immediate questions is the function of Oct4 in pluripotent cells that have already established X inactivation. Human embryonic stem cells and epiblast derived stem cells from mice have already undergone X inactivation despite expressing high levels of Oct4 [10, 11]. Explaining Oct4 function in these different cellular contexts will require the introduction of additional regulatory components. Also the function of the Oct4 binding site in the first intron of *Xist* is not fully understood. This binding site has been recently found to exert repression of *Xist* independent of *Tsix* transcription [7]. This is entirely consistent with its position well within the *Tsix* transcription unit quite distant to the *Tsix* promoter, which is also observed by Donohoe et al. but not further examined. It appears likely that Oct4 contributes to *Xist* repression in *Tsix* dependent and independent modes. Ultimately, deletion of the Oct4 binding site in *Xist* intron 1 will be required for dissecting its contribution to the regulation of X inactivation. Albeit certain details of the mechanism remain to be elucidated, the data presented by Donohoe *et al*. make a strong case for Oct4 as an autosomal factor for *Xist* regulation.

Less clear is the X-chromosomal determinant that enables X inactivation in female cells. Presently, data indicate that the pairing of *Xic* regions could perform this function to activate *Xist* expression. However, a recent study has demonstrated that an X chromosome lacking *Xic* sequences including *Tsix* can still be counted and will trigger X inactivation of the other wild type X chromosome [12]. This strongly argues for an X-linked activator of X-inactivation that resides outside the *Xist*-*Tsix* region. This activator remains to be identified. The scenario is further complicated by the fact that a region quite distant from *Xist* and *Tsix* has been shown to lead to pairing of the X chromosomes long before initiation of X inactivation [13]. How this pairing is established and if it is also dependent on Oct4 and CTCF is not known at present. Identification of Oct4 as a regulator of X inactivation presents a major step forward to address these important questions.

References

1 Lee JT, Davidow LS, Warshawsky D. Tsix, a gene antisense to Xist at the X-inactivation centre. *Nat Genet* 1999; **21**:400-404.
2 Lee JT, Lu N. Targeted mutagenesis of Tsix leads to nonrandom X inactivation. *Cell* 1999; **99**:47-57.
3 Bacher CP, Guggiari M, Brors B, *et al.* Transient colocalization of X-inactivation centres accompanies the initiation of X inactivation. *Nat Cell Biol* 2006; **8**:293-299.
4 Xu N, Tsai CL, Lee JT. Transient homologous chromosome pairing marks the onset of X inactivation. *Science* 2006; **311**:1149-1152.
5 Xu N, Donohoe ME, Silva SS, Lee JT. Evidence that homologous X-chromosome pairing requires transcription and Ctcf protein. *Nat Genet* 2007; **39**:1390-1396.
6 Donohoe ME, Zhang LF, Xu N, Shi Y, Lee JT. Identification of a Ctcf cofactor, Yy1, for the X chromosome binary switch. *Mol Cell* 2007; **25**:43-56.
7 Navarro P, Chambers I, Karwacki-Neisius V, *et al.* Molecular coupling of Xist regulation and pluripotency. *Science* 2008; **321**:1693-1695.
8 Ying QL, Wray J, Nichols J, *et al.* The ground state of embryonic stem cell self-renewal. *Nature* 2008; **453**:519-523.
9 Donohoe ME, Silva SS, Pinter SF, Xu N, Lee JT. The pluripotency factor Oct4 interacts with Ctcf and also controls X-chromosome pairing and counting. *Nature* 2009; **460**:128-132.
10 Silva SS, Rowntree RK, Mekhoubad S, Lee JT. X-chromosome inactivation and epigenetic fluidity in human embryonic stem cells. *Proc Natl Acad*

Sci USA 2008; **105**:4820-4825.

11 Guo G, Yang J, Nichols J, *et al.* Klf4 reverts developmentally programmed restriction of ground state pluripotency. *Development* 2009; **136**:1063-1069.

12 Monkhorst K, Jonkers I, Rentmeester E, Grosveld F, Gribnau J. X inactivation counting and choice is a stochastic process: evidence for involvement of an X-linked activator. *Cell* 2008; **132**:410-421.

13 Augui S, Filion GJ, Huart S, *et al.* Sensing X chromosome pairs before X inactivation via a novel X-pairing region of the Xic. *Science* 2007; **318**:1632-1636.