Disentangling the X chromosome inactivation network

Edda G. Schulz[1], Johannes Meisig[2], Nils Blüthgen[2] and Edith Heard[1]

Random X-chromosome inactivation (XCI) during early embryogenesis of female mammals acts to equalize the gene dosage between the sexes and is initiated by upregulation of the *Xist* gene. We have developed a stochastic model of *Xist's* regulatory network and found that female-specific monoallelic expression of *Xist* requires (1) a cis-acting positive feedback loop acting as a switch, (2) an X-linked *Xist* activator and (3) a trans-acting negative feedback loop. Moreover, we propose the existence of a so far unknown checkpoint, which ensures that differentiation only proceeds after one X has been inactivated. In summary, we present an experiment-based model of how female-specific monoallelic expression of Xist is achieved in a developmental context.

***Keywords* — X chromosome inactivation, stochastic modeling, gene-regulatory networks, single-cell analysis**

I. Background

To equalize the gene dosage between the sexes, one X-chromosome is inactivated in female mammals early during embryonic development. The inactive state is then stably propagated during cell divisions [1]. XCI is usually random and is initiated by the up-regulation of the non-coding Xist RNA from the future inactive X, which then silences the chromosome *in cis*. A network of *cis*- and *trans*-acting factors controls Xist up-regulation, and limits its expression to one chromosome. The structure of *Xist's* regulatory network and the mechanism through which female-specific monoallelic expression of Xist is ensured in the context of differentiation, remain elusive however.

II. Female-specific monoallelic Xist expression

In the first step, we attempted to identify the simplest network that can account for monoallelic upregulation of Xist, restricted to a female (XX) context. We have developed a series of stochastic models, which were simulated over a large range of parameter values. We identified three network motifs that are strictly required to account for the experimentally observed expression pattern of Xist: A *cis*-acting positive feedback loop acting as a switch, an X-linked *Xist* activator to ensure XX-specific expression and a trans-acting negative feedback loop that is required for monoallelic expression.

The resulting model makes a number of predictions that have been tested experimentally by performing single-cell measurements of known members of the Xist regulatory network [2-4]. Moreover, characterized mutations affecting Xist expression are simulated and compared to the experimentally observed phenotype.

III. Developmental *Xist* regulation

X inactivation occurs during a defined time window in the early embryo and can be recapitulated in differentiating female embryonic stem (ES) cells. Pluripotency factors, which are expressed in undifferentiated ES cells, where they are thought to repress *Xist* [3, 5], are downregulated during differentiation and have therefore been proposed as the developmental trigger of Xist upregulation. This model would predict a negative correlation between Xist and pluripotency factor levels at the single cell level during the onset of XCI. Experimental analysis of this relationship, however, revealed only a minor, if any anti-correlation, suggesting the involvement of so far unidentified factors. To further analyze the link between Xist expression and differentiation, we performed a kinetic transcriptome analysis during early differentiation of male and female ES cells. To our surprise, we found that male and female cells differ strongly in their differentiation speed. We propose the existence of a so far unidentified checkpoint, whereby differentiation is blocked as long as two X chromosomes in a cell are active. This could be due to an X-linked gene that only prevents differentiation when present in a double dose. We are currently testing this hypothesis and trying to identify the responsible gene.

Acknowledgements: This work was funded by Epigenesys, Syboss, HFSP.

[1]Mammalian Developmental Epgenetics Group, Institut Curie, Paris, France E-mail: Edda.Schulz@curie.fr

[2]Institute of Pathology, Charité and Institute of Theoretical Biology, Humboldt Universität, Berlin, Germany. E-mail: Nils.Bluethgen@charite.de

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