

Nuclear and Chromosome Dynamics during X-Chromosome Inactivation and Development

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In female mammals, one of the two X chromosomes is converted from the active euchromatic state into inactive heterochromatin during early embryonic development. This process, known as X-chromosome inactivation, results in the transcriptional silencing of over a thousand genes and ensures dosage compensation between the sexes. X inactivation is a dramatic example of mammalian epigenetics, involving differential regulation of two homologous chromosomes within the same nucleus, in a mitotically heritable but developmentally reversible manner. We are interested in the mechanisms and kinetics of this process in early mouse embryos and differentiating embryonic stem (ES) cells. Given the monoallelic character of X inactivation, we are particularly interested in the potential role of sub-nuclear compartmentalization in this process, both at the level of the master control locus of X inactivation, the *Xic*, and the non-coding *Xist* transcript it produces, that is responsible for inducing transcriptional silencing in *cis*. Our recent studies using both RNA/DNA FISH and live cell imaging approaches (Augui et al, 2007; Masui et al, 2011) have shown that during female ES cell differentiation, the two *Xics* come into proximity of each other transiently just prior to the initiation of X inactivation and that this could participate in the mechanism through which an XX cell ensures the monoallelic up-regulation of *Xist*. We have also demonstrated that *Xist* RNA may have a role in spatially segregating and reorganizing the X chromosome in the nucleus during X inactivation (Chaumeil et al, 2006; Chow et al, 2010). Our current work is focused on examining the detailed chromosome structure of the X chromosome, before and after X inactivation, using both chromosome conformation capture techniques (4C and 5C) as well as high resolution structured illumination microscopy (SIM).

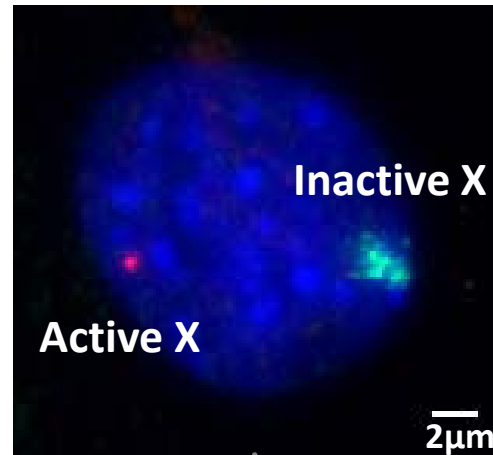
Masui O., Bonnet I., Le Baccon P., Brito I., Pollex T., Murphy N., Hupé P., Barillot E., Belmont A. and Heard E. (2011) Live cell chromosome dynamics and outcome of X-chromosome pairing events during ES cell differentiation. *Cell* 145: 447-458.

Chow, J., Ciaudo, C., Fazzari, M., Mise, N., Servant, N., Glass, J.L., Attreed, M., Avner, P., Wutz, A., Barillot, E., Grealley, J.M., Voinnet, O. and Heard, E. (2010) LINE1 activity in facultative heterochromatin formation during X-chromosome inactivation. *Cell* 141: 956-969.

-Augui, S., Fillion, G., Huart, S., Guggiari, M., Maresca, M., Stewart, F. and Heard, E. (2007) Sensing X-chromosome pairs prior to X inactivation via a novel X-pairing region of the *Xic*. *Science* 318, 1632-1636.

Chaumeil, J., Le Baccon, P., Wutz, A. and Heard E. (2006) A novel role for *Xist* RNA in the formation of a repressive nuclear compartment into which genes are recruited when silenced. *Genes and Development* 20: 2223-2237.

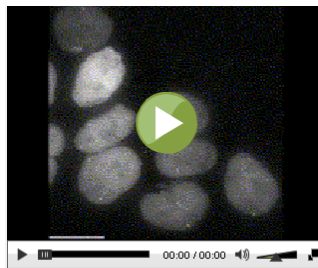
Gaining insights into chromosome structure and nuclear organisation using the X chromosome as a model



Xist RNA
AtrX RNA
DNA

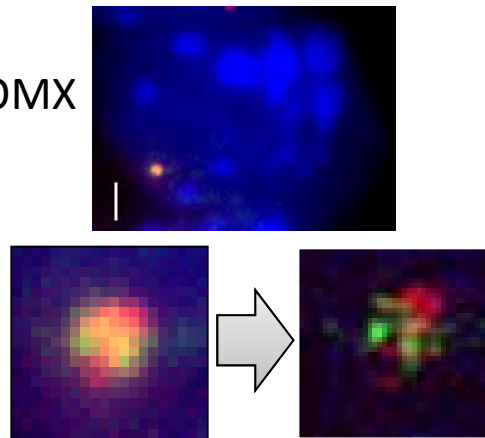
Live-cell imaging of chromosome dynamics

TetO/TetR tagging



High-resolution microscopy

OMX



Chromosome Conformation Capture

