The role of CTCF and chromosome topology in the regulation of gene expression in imprinted domains.

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Project description

The two metres of DNA in mammalian cells are exquisitely folded within the nucleus. Chromosomes are organised along their length into compartments, topologically associated domains (TADs) and contact loops[1]. TADs are self-interacting regions that are demarcated by the binding of the insulator protein CTCF and are thought to constrain gene regulation to within a specific region.

Genomic imprinting is an epigenetically controlled process whereby genes are expressed in a parent-of-origin specific manner. Many imprinted genes are found in clusters in the genome where they are controlled by cis-acting elements that determine the allele-specific expression of all the genes in the domain[2]. The range of these cis-acting elements is likely determined by local chromosome topology and many imprinted regions are known to show parent-of-origin specific conformations[3,4].

The *Dlk1/Gtl2* imprinting domain extends for 1.25 Mb on mouse chromosome 12. Recently, several studies have suggested that weak paternally biased genes are located at either side of the domain potentially extending the imprinted cluster to 2.46 Mb[5,6]. We have verified weak paternal bias in two genes on the proximal side of the canonical imprinted domain, *Wdr25* and *Wars*, in the postnatal brain. Interestingly, we have also identified a maternal chromosome-specific TAD in the Dlk1/Gtl2 domain. This TAD extends between two CTCF binding sites in the maternally expressed *Gtl2* gene and two CTCF sites in *Wdr25* (Edwards and Ferguson-Smith unpublished). To investigate the functional relevance of this 3D topology, and to see if it affects the weak parental-origin specific biases at the boundary, we have knocked out the CTCF sites in *Gtl2* and *Wdr25*. We find loss of the *Gtl2* CTCF sites results in changes to expression and imprinting in the cluster. This project aims to assess the role of the *Wdr25* CTCF sites, at the other side of the interaction, in regulating gene expression within and surrounding the imprinted cluster. It will also address whether these CTCF sites act as a physical barrier to imprinting spreading out of the canonical domain.

Reciprocal crosses of *Wdr25*-CTCF mutant mice with wildtype *Mus musculus castaneus* have been performed and five subregions of the brain have been dissected at postnatal day seven. The student will extract RNA from these tissues, generate cDNA and perform quantitative PCR to assess expression levels in maternal and paternal heterozygotes and their wildtype littermates. The imprinting status of each of the genes will then be established by allele specific pyrosequencing across known polymorphisms. Time allowing, the student will also make libraries to investigate changes to local topology by 4Cseq. The results from these experiments will be compared with those from mice lacking the *Gtl2* CTCF sites to build up a picture of the role of parent-of-origin specific topology on gene regulation in imprinted domains.

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2. Edwards, C. A. & Ferguson-Smith, A. C. Mechanisms regulating imprinted genes in clusters. *Curr Opin Cell Biol* **19**, 281–289 (2007).
3. Murrell, A., Heeson, S. & Reik, W. Interaction between differentially methylated regions partitions the imprinted genes Igf2 and H19 into parent-specific chromatin loops. *Nat Genet*

36, 889–893 (2004).
4. Tan, L. *et al.* Changes in genome architecture and transcriptional dynamics progress independently of sensory experience during post-natal brain development. *Cell* (2021) doi:10.1016/j.cell.2020.12.032.
5. Bonthuis, P. J. *et al.* Noncanonical genomic imprinting effects in offspring. *Cell Rep.* **12**, 979–991 (2015).
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Short summary

The DNA of cells is exquisitely folded within the nucleus. Topologically associated domains (TADs) are self-interacting regions on a chromosome that are demarcated by CTCF binding and thought to constrain gene regulation to within the TAD.

This project aims to study the role of topology in the control of gene expression using genomic imprinting as a model. We will explore the impact of deleting CTCF sites at the edge an imprinting domain on gene expression, imprinting and topology.

Key aims and tasks:

i) Extract RNA from tissues dissected from mice heterozygous for CTCF binding site deletion.
ii) Perform quantitative PCR to assess expression levels in maternal and paternal heterozygotes and their wildtype littermates.
iii) Assess imprinting using allele specific pyrosequencing across known polymorphisms then compare these results with those from other models.
iv) Use circular chromosome conformation capture sequencing (4Cseq) to assess changes to topology in the region.

Skills required: Basic knowledge of molecular biology and genetics. Some lab experience is desirable but full training will be given.

In person: The project is lab based but some analysis can be done remotely.

Duration: This is an 8 week project.