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CHROMATIN

Going a TAD out on a limb

New findings reveal how genomic duplications can result in different phenotypes, depending on their size and localization, by restructuring the higher-order chromatin structure to form new topologically associated domains (TADs), which the authors name neo-TADs. The study also shows that the effect of TADs on gene regulation depends on their integrity in relation to neighbouring TADs and genes.

Using chromosome conformation capture (capture Hi-C and 4C-seq methods), Franke *et al.* investigated the effects of genomic duplications in the *SOX9* locus, a region on chromosome 17q24 in humans that has previously been associated with a number of disorders and malformations.

The *SOX9* locus is compartmentalized into two large TADs, one containing the *SOX9* gene and the other containing two genes encoding potassium channels, *KCNJ2* and *KCNJ16*. Genome-wide profiling of chromatin contacts using Hi-C showed that duplications associated with female-to-male sex reversal are located within the *SOX9*-containing TAD (intra-TAD duplications). By contrast, two other types of duplication of differing size encompass the same region but extend further upstream into the neighbouring TAD containing *KCNJ2* and *KCNJ16* (inter-TAD duplications), and result in either no phenotype or the congenital limb malformation Cooks syndrome.

Comparing allele-specific 4C-seq profiles of patient fibroblasts with intra-TAD or inter-TAD duplications with those of controls, the authors found that intra-TAD duplications altered the interaction profile but had no effect on overall chromatin structure, whereas the inter-TAD duplicated region contacted primarily itself, resulting in the formation of a new domain.

Franke *et al.* then genetically modified mice using CRISPR–Cas9 genome editing and the Cre–*loxP* system to generate an intra-TAD duplication (mimicking the human sex reversal mutation) or an inter-TAD duplication (equivalent to the human duplication with no phenotype), respectively. Capture Hi-C profiles confirmed the *in vitro* findings that intra-TAD duplications do not disrupt higher-order structure, but that the duplication spanning across two TADs and their boundary resulted in the formation of a neo-TAD that was insulated from neighbouring TADs.

The regulatory potential of the neo-TAD was determined in mutant mice by comparing the expression of a *lacZ* reporter gene contained within the inter-TAD duplication with that of *lacZ* reporters inserted in the TADs comprising *Sox9* and the *Kcnj* genes. This revealed that the regulatory sequence within the neo-TAD was functional and capable of driving expression in a tissue-specific *Sox9* expression pattern.

Using *trans*-allelic recombination the team then generated mice with a duplication extending towards the region containing *Kcnj2* and *Kcnj16*, similar to human Cooks syndrome, which resulted in the incorporation of these genes into the neo-TAD. The inclusion of *Kcnj2* in the neo-TAD led to ectopic contacts between *Kcnj2* and parts of the *Sox9*-containing TAD, resulting in increased *Kcnj2* expression in a *Sox9* expression-like pattern, as determined by *in situ* hybridization and confirmed by RNA sequencing. Importantly, altered *Kcnj2* expression was accompanied by major phenotypic changes, with mice heterozygous for the inter-TAD duplication exhibiting limb malformations resembling human Cooks syndrome. Of note, deletion of a boundary alone increased interactions between TADs but had no major effect on TAD structure.

The finding that genes that become incorporated into a newly formed TAD can be activated by its regulatory elements, leading to pathogenic effects, has great clinical implications. Future work will need to establish the feasibility of predicting phenotype from genomic duplications identified, for example, during diagnostic screening.

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ORIGINAL ARTICLE Franke, M. *et al.* Formation of new chromatin domains determines pathogenicity of genomic duplications. *Nature* http://dx.doi.org/10.1038/nature19800 (2016)
FURTHER READING Bonev, B. & Cavalli, G. Organization and function of the 3D genome. *Nat. Rev. Genet.* http://dx.doi.org/10.1038/nrg.2016.112 (2016)