
Credit: Mike Lane/Alamy

maintain sustained periods of physical activity, such as is needed during hunting. *AGT* encodes the precursor of a number of peptides, including angiotensin II (AngII); as a component of the renin–angiotensin system, AngII is involved in the cardiovascular response to aerobic activity.

Finally, a number of genes involved in haemostasis (the regulation of blood loss) were also found to be under positive selection. These genes are involved in blood coagulation and in platelet aggregation, activation and spreading. Komodo dragons engage in aggressive battles over food, territories and mates. Given that their saliva has anticoagulant and hypotensive effects, the authors suggest that positive selection acting on the coagulation system may result in protection from conspecific bite wounds obtained during these conflicts.

The Komodo dragon genome has provided insight into genes that underpin important biological adaptations in monitor lizards. It will also be a valuable resource for furthering our understanding of the biology of the estimated 10,000 highly diverse species of reptiles found worldwide.

Dorothy Clyde

ORIGINAL ARTICLE Lind, A. L. et al. Genome of the Komodo dragon reveals adaptations in the cardiovascular and chemosensory systems of monitor lizards. *Nat. Ecol. Evol.* **3**, 1241–1252 (2019)

according to their genetic interaction profiles to build a gain-of-function genetic interaction map from the fitness measurements.

To determine underlying mechanisms, a subset of overexpression genetic interactions (132 gene pairs) were profiled transcriptionally using Perturb-seq. Single-gene perturbations were also profiled for direct comparison with gene-pair perturbations. Transcriptional readouts totalled 287 perturbations measured across ~110,000 single cells in one pooled experiment.

The transcriptional phenotype measurements provided by Perturb-seq were used to define a high-dimensional surface, which the authors call a genetic interaction manifold, with each point representing a possible cellular transcriptional state. This map was visualized by dimensionality reduction to a plane. While genetic interactions may result in similar outcomes when viewed at the fitness level (for example, growth arrest), the high-dimensional perspective shows that these genetic interactions can lie in markedly different parts of the genetic interaction manifold, which indicates that the molecular mechanisms underlying the fitness outcome differ. Conversely, clusters of transcriptionally similar perturbations in the manifold can reflect common mechanistic underpinnings. For example, one cluster of mean expression profiles comprised canonical cell cycle regulators; hence, the single-cell data confirmed that the growth defect seen at the fitness level arose because these gene-pair perturbations all induced cell cycle arrest.

The approach showcased by Norman, Horlbeck et al. has the potential to enable "large-scale searches for synthetic lethal interactions in cancer, the discovery of gene targets that lessen the severity of genetic disease, and, more generally, the understanding of how complex, multigenic interactions govern biological traits and disease risk," conclude the authors.

Linda Koch

ORIGINAL ARTICLE Norman, T. M. et al. Exploring genetic interaction manifolds constructed from rich single-cell phenotypes. *Science* https://doi.org/10.1126/science.aax4438 (2019)

GENE EXPRESSION

Toppling TAD tenets

Developmental enhancers are typically located in the same topologically associating domain (TAD) as their target genes, and the prevailing notion has been that the merger or rearrangement of TADs leads to ectopic gene expression, as shown at some loci. However, whether all genes are affected in a similar manner was unclear. Now, a study in *Nature Genetics* shows that genomic rearrangements that cause extensive changes to chromatin topology do not alter expression for the majority of genes, suggesting that properties other than chromatin topology determine enhancer–promoter interactions.

In vertebrates, CTCF binding is enriched at the boundaries of TADs. As CTCF has been shown to act as an insulator in both insulator assays in *Drosophila melanogaster* and at imprinted loci, it has been assumed that TAD boundaries act as insulators that block enhancers from interacting with genes on the other side of the boundaries. "That TAD boundaries restrict enhancer function, that is, that enhancers can only regulate genes in the same TAD and not 'cross' the boundary, has since almost become a dogma," says senior author Eileen Furlong (EMBL Heidelberg, Germany).

To assess this question more systematically, Ghavi-Helm et al. chose the *D. melanogaster* balancer chromosomes as a source of genome rearrangements. These highly rearranged chromosomes, generated mainly by X-ray irradiation, can be used to genetically screen a model organism population and select for heterozygotes carrying a lethal recessive mutation. The team crossed a balancer strain to an isogenic wild-type strain and then performed allele-specific Hi-C and RNA sequencing (RNA-seq) to determine the impact on topology and gene expression of both the balancer and the wild-type chromosome.

Strikingly, the researchers found that while TAD boundaries mattered for some genes, as shown previously, these instances were rare. In the majority of cases, gene expression was not affected by genomic rearrangements even though many TADs were reshuffled, sometimes doubling in size. In other words, enhancers still regulated their correct genes despite other genes now being in their vicinity. "This finding is very surprising to many, but it actually fits with recent emerging data from others," says Furlong. For example, previously "published imaging data show huge cell-to-cell heterogeneity in TAD size, so the location of the 'boundary' is different and therefore cannot be the key to restraining enhancer activity, otherwise gene expression would vary greatly from cell to cell."

The notion that you can massively disrupt genome organization without large-scale effects on gene expression is important, as it emphasizes that TADs are not essential for regulating the expression of all genes. How far these findings can be extrapolated considering that the original balancer inversions were viable needs to be determined. Regardless, it is evident that many lessons remain to be learned about the relationship between chromatin topology and gene expression.

Linda Koch

ORIGINAL ARTICLE Ghavi-Helm, Y. et al. Highly rearranged chromosomes reveal uncoupling between genome topology and gene expression. *Nat. Genet.* https://doi.org/10.1038/s41588-019-0462-3 (2019)
FURTHER READING Schoenfelder, S. & Fraser, P. Long-range enhancer–promoter contacts in gene expression control. *Nat. Rev. Genet.* **20**, 437–455 (2019)