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Examining Topological Domain Influence on Enhancer Function

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Summary

Enhancers regulate the expression of target genes across large genomic distances, but it is unclear how recently discovered topological domains affect this regulation. Reporting in *Developmental Cell*, Symmons et al. (2016) show that the endogenous *Shh* topological domain promotes functional interactions between *Shh* and its remote enhancer.

Main text

Topological domains (TADs) consist of continuous genomic regions which preferentially contact themselves more than neighbouring regions when folded in the nucleus (Dixon et al., 2012; Nora et al., 2012; Sexton et al., 2012). TAD boundaries are highly conserved between cell types, and have been proposed as a fundamental structural unit of chromatin folding (Dixon et al., 2012). Since their discovery, there has been much interest in whether and how TADs influence transcriptional regulation, in particular through controlling the activity of regulatory DNA elements known as enhancers. Enhancers are often located far from their target genes in the linear genome, and there is a

growing body of evidence that chromatin interactions bring enhancers into close spatial contact with their target genes and that these interactions or "loops" can be important for gene activation.

Previous work from Spitz and colleagues has shown that TADs can correspond to regulatory domains (Symmons et al., 2014). In addition, sequence variants that remove or rearrange TAD boundaries can lead to enhancer hijacking, where genes that are normally in an adjacent TAD become regulated by inappropriate enhancers (Franke et al., 2016; Lettice et al., 2011; Lupiáñez et al., 2015; Northcott et al., 2014), arguing that TAD boundaries can act as insulators, restricting enhancer activity to only those genes within the same TAD. One unresolved question is how enhancers achieve specificity: can they act pervasively on any gene within a given TAD or are their target genes selected on some other basis? Spitz and colleagues now show that TADs can actively contribute to gene expression regulation by minimizing the effect of genomic distance between enhancers and their target genes.

Symmons et al. (2016) present a detailed investigation of how the positions of the sonic hedgehog gene (*Shh*) and its well characterized limb enhancer (the ZRS) affect gene expression in the context of the surrounding TADs. They begin by analysing the expression patterns of a reporter gene inserted at one of 59 different positions across the *Shh* locus, finding that most insertions that fall within the *Shh* TAD show expression in the developing limb of the mouse, whilst most insertions outside of this TAD do not. Since the ZRS is the sole enhancer responsible for *Shh* expression in the limb, they conclude that the ZRS is able to activate gene expression pervasively within the encompassing TAD, in a manner unrestricted by genomic distance (Fig. 1a). To test this hypothesis, they engineer a series of deletion or duplication alleles which decrease or increase the distance between the ZRS and the endogenous *Shh* gene, finding that these structural changes do not disrupt *Shh* expression in the limb.

Having investigated the range of ZRS activity within the endogenous TAD, Symmons et al. (2016) go on to examine the effect of disrupting the wild type *Shh* TAD structure. Genomic inversions which include one TAD boundary are found to disrupt the formation of a compact TAD at the *Shh* locus as well as abrogating *Shh* expression in the limb, whereas *Shh* function is unaffected by inversions that keep the TAD intact. Interestingly, TAD-disrupting inversions which reduce the genomic distance between *Shh* and the ZRS show progressively less disrupted limb morphology, possibly indicating an effect of genomic distance in the partial rescue of ZRS-driven *Shh* expression in the limb. This finding has fascinating implications for the field, as it suggests that enhancer function is determined by genomic distance in the absence of a compact TAD (Fig. 1b), whilst the endogenous *Shh* TAD structure is able to buffer against genomic distance effects.

The results raise a number of important questions; first and foremost whether "distance buffering" is a general property of TADs, or whether it applies only in specific circumstances. Another key observation is that ZRS activity within the *Shh* TAD is not uniform: there are a number of reporter gene insertions which show no activity in the developing limb (Fig. 1a). What genomic features, local

or global, prevent activation? What other factors can predict responsiveness to an enhancer? Spitz and colleagues show that the strength of interactions between insertion positions and the ZRS correlates with expression in the limb, suggesting that chromatin within the *Shh* TAD might be folded in a way that isolates some regions from the ZRS - but this effect only explains some of the variability and other factors may well be important. If intra-TAD folding determines which insertions are responsive to the ZRS, some insertions which are unresponsive in the endogenous TAD might become activated after TAD disruption. Regardless, increasing evidence now points towards a key role for genome folding at the level of TADs in enhancer function.

References

Dixon, J.R., Selvaraj, S., Yue, F., Kim, A., Li, Y., Shen, Y., Hu, M., Liu, J.S., Ren, B., 2012. Nature *485*, 376–380.

Franke, M., Ibrahim, D.M., Andrey, G., Schwarzer, W., Heinrich, V., Schöpflin, R., Kraft, K., Kempfer, R., Jerković, I., Chan, W.-L., et al., 2016. Nature *538*, 265–269.

Lettice, L.A., Daniels, S., Sweeney, E., Venkataraman, S., Devenney, P.S., Gautier, P., Morrison, H., Fantes, J., Hill, R.E., FitzPatrick, D.R., 2011. Hum. Mutat. *32*, 1492–1499.

Lupiáñez, D.G., Kraft, K., Heinrich, V., Krawitz, P., Brancati, F., Klopocki, E., Horn, D., Kayserili, H., Opitz, J.M., Laxova, R., et al., 2015. Cell *161*, 1012–1025.

Nora, E.P., Lajoie, B.R., Schulz, E.G., Giorgetti, L., Okamoto, I., Servant, N., Piolot, T., van Berkum, N.L., Meisig, J., Sedat, J., et al., 2012. Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature.

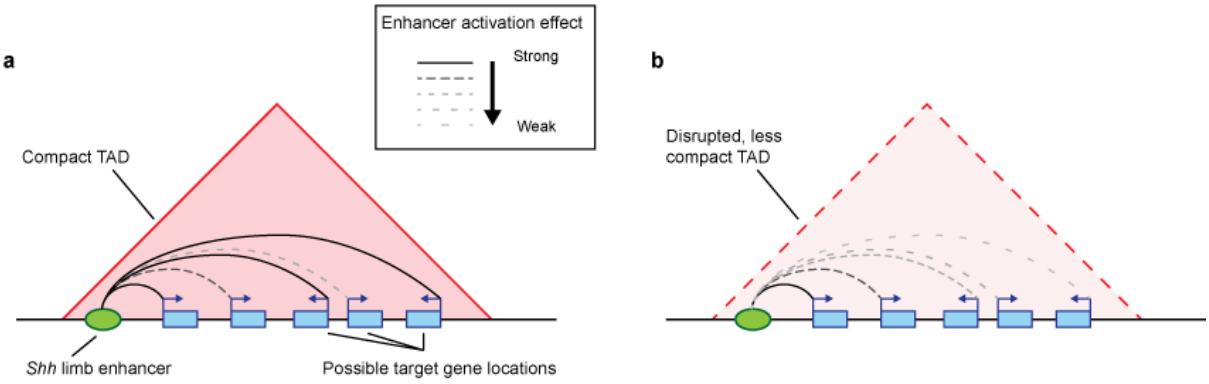
Northcott, P.A., Lee, C., Zichner, T., Stütz, A.M., Erkek, S., Kawauchi, D., Shih, D.J.H., Hovestadt, V., Zapatka, M., Sturm, D., et al., 2014. Nature *511*, 428–434.

Sexton, T., Yaffe, E., Kenigsberg, E., Bantignies, F., Leblanc, B., Hoichman, M., Parrinello, H., Tanay, A., Cavalli, G., 2012. Cell *148*, 458–72.

Symmons, O., Uslu, V.V., Tsujimura, T., Ruf, S., Nassari, S., Schwarzer, W., Ettwiller, L., Spitz, F., 2014. Genome Res. *24*, 390–400.

Symmons, O., Pan, L., Remeseiro, S., Aktas, T., Klein, F., Huber, W., Spitz, F., 2014. Dev. Cell. ....*?*

Figures



a, The formation of a compact topological domain (TAD) enables the *Shh* limb enhancer to activate gene expression across very large genomic distances. Although enhancer activity is pervasive throughout the TAD, it is not uniform. Genes located in certain "cold spots" are less affected by the activity of the enhancer, either due to specific folding of chromatin within the TAD or due to local chromatin effects. **b**, When the surrounding TAD is disrupted and made less compact (e.g. by a genomic inversion encompassing one of the TAD boundaries), the activity of the limb enhancer becomes dependent on the genomic distance between the enhancer and a target gene.