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GENE EXPRESSION

# Developmental enhancers in action

Enhancers are distal genetic elements that regulate cell-type specific gene expression. Three recent papers provide new insights into the functional properties of enhancers during development.

The number of predicted enhancers is about tenfold the number of genes; it remains unclear whether this represents regulation of gene expression in an additive and/or in a redundant manner. Osterwalder *et al.* used the mouse developing limb to study enhancer function during morphogenesis. Individually deleting ten conserved enhancers of genes associated with mouse and human congenital limb malformation caused no significant change in target-gene expression and, importantly, no limb abnormalities. This indicated that many conserved limb enhancers are not individually essential for limb morphogenesis. The selected panel of enhancers included three enhancer pairs with overlapping limb activity and the same predicted target gene. In two out of three cases, embryos with homozygous deletions of the enhancer pair showed reduction in target-gene expression and limb abnormalities.

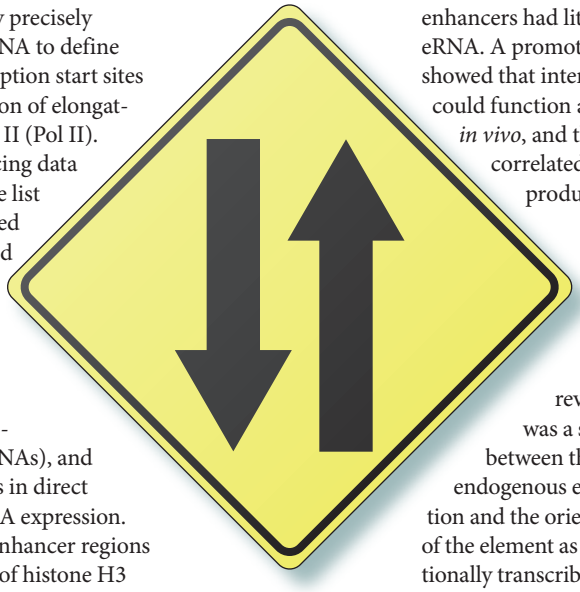
For each of the two target genes, the authors generated compound heterozygous mice harbouring one or two disrupted enhancers with a wild-type gene on one allele and a disrupted gene (with wild-type enhancers) on the other allele. In each case, deletion of one enhancer exacerbated the mild heterozygous phenotype, and deletion of both enhancers caused a severe limb phenotype comparable to the homozygous gene loss phenotype. Thus, in a genetic sensitized background such as gene heterozygosity, the two enhancers functioned in an additive manner and both were required for proper morphogenesis.

Henriques *et al.* used *Drosophila melanogaster* S2 cells to study transcription at enhancers, which leads to the generation of enhancer RNAs (eRNAs). They precisely sequenced nascent RNA to define unannotated transcription start sites (TSSs) and the position of elongating RNA polymerase II (Pol II). Crossing the sequencing data with a comprehensive list of functionally-defined enhancer loci revealed that 49% of the unannotated TSSs fell within enhancer loci. Furthermore, functional enhancers highly expressed TSS-associated RNAs (eRNAs), and enhancer activity was in direct correlation with eRNA expression. Highly-transcribed enhancer regions exhibited high levels of histone H3 Lys4 trimethylation (H3K4me3) rather than the previously reported H3K4me1.

Analysis of enhancer transcription revealed that, similarly to promoter-proximal pausing, Pol II paused at enhancers, although in a more transient manner. Pol II typically did not proceed with transcription beyond pausing, or more than ~100 nucleotides from the enhancer TSS, and a higher percentage of eRNAs than of mRNAs were oligo-adenylated, suggesting higher levels of transcription termination and RNA degradation at enhancers than at promoters.

The authors also showed in mouse cells that pausing at super-enhancers and at their associated genes is particularly transient and facilitates high expression levels of the target genes; consequently, the expression of these genes is not reliant on pausing factors and is even delayed by Pol II pausing.

Mikhaylichenko *et al.* examined the relationship between transcription directionality and enhancer and promoter function in *D. melanogaster* embryos. Like Henriques *et al.*, they found that transcription at enhancers was indicative of enhancer activity, although, importantly, some active enhancers had little or no detectable eRNA. A promoter reporter assay showed that intergenic enhancers could function as weak promoters *in vivo*, and that this capacity correlated with eRNA production levels.



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> “the two enhancers functioned in an additive manner”

In embryos the use of a reporter construct assessing enhancer and promoter activity simultaneously revealed that there was a strong correlation between the directionality of endogenous enhancer transcription and the orientation functionality of the element as a promoter: bidirectionally transcribed enhancers mostly showed orientation-independent promoter activity, whereas unidirectionally transcribed enhancers supported orientation-dependent promoter function in the same direction. Similarly, bidirectional promoters could function as developmental enhancers, albeit probably only for the same gene for which they serve as promoters and when transcription is initiated from an alternative promoter. Again, there was strong correlation between the transcription directionality of the promoter and the orientation of its enhancer activity.

Together, the three papers establish the complexity and diversity of function of developmental enhancers, and the relationship between their function and their transcriptional properties.

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**ORIGINAL ARTICLES** Osterwalder, M. *et al.* Enhancer redundancy provides phenotypic robustness in mammalian development. *Nature* https://doi.org/10.1038/nature25461 (2018) | Henriques, T. *et al.* Widespread transcriptional pausing and elongation control at enhancers. *Genes Dev.* https://doi.org/10.1101/gad.309351.117 (2018) | Mikhaylichenko, O. *et al.* The degree of enhancer or promoter activity is reflected by the levels and directionality of eRNA transcription. *Genes Dev.* https://doi.org/10.1101/gad.308619.117 (2018)

**FURTHER READING** Heinz, S. *et al.* The selection and function of cell-type specific enhancers. *Nat. Rev. Mol. Cell Biol.* **16**, 144–154 (2015)