Gene regulation

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Enhancer contacts during embryonic development show diverse interaction modes and modest yet significant increases upon gene activation

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The exact relationship between 3D chromatin interactions and enhancer function is unclear. By probing three-dimensional enhancer interactions in developing embryos, two studies now show nuanced dynamics in tissue-specific contexts and reveal how moderately increased enhancer–promoter interactions coincide with functionality.

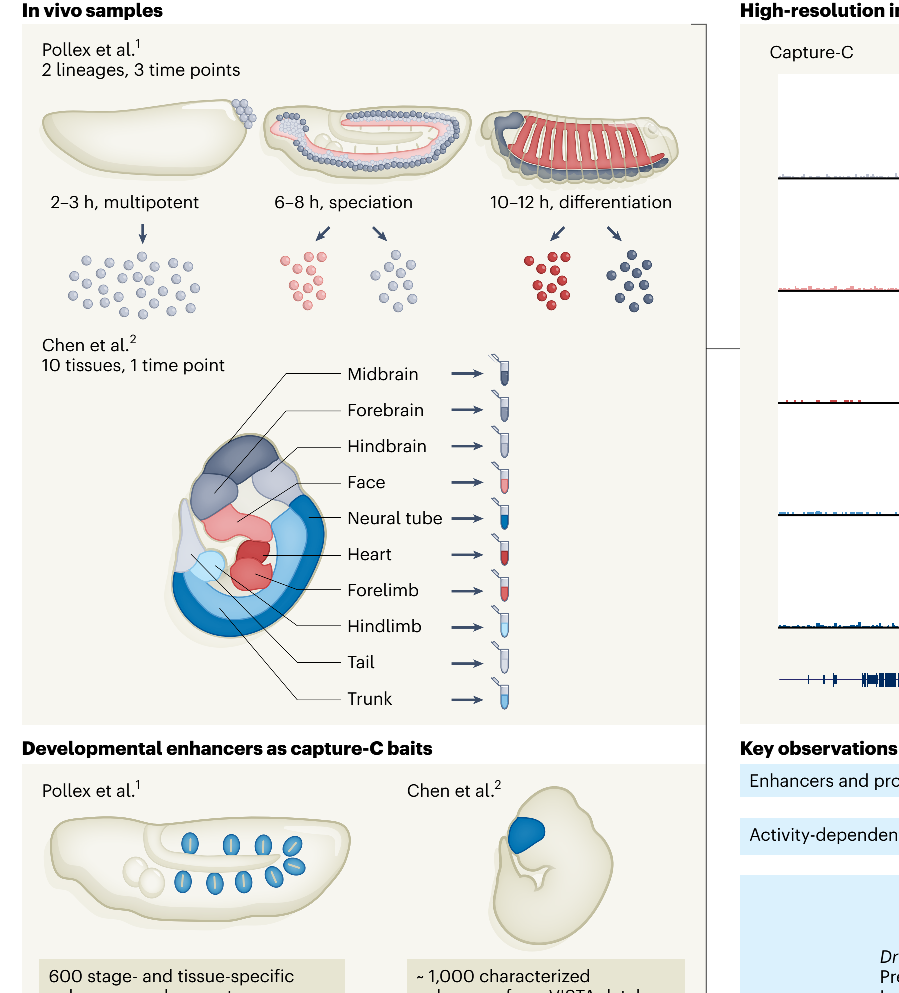
Cell type-specific gene expression is controlled by *cis*-regulatory elements known as enhancers that can reside at large genomic distances from their target genes. To exert their function (that is, to activate or regulate transcription), enhancers come into physical proximity with their target gene promoters. How such enhancer–promoter interactions relate to gene expression has been intensively studied, especially after the widespread adoption of chromatin conformation capture-based (3C) assays that enable the determination of physical interaction frequencies between linearly distant genomic regions. In this issue of *Nature Genetics*, two studies by Pollex et al.[1] and Chen et al.[2] use a 3C-derivative approach known as capture-C to profile the chromatin interactions of hundreds of enhancers during embryonic development and investigate their regulatory relationships.

Single-locus mechanistic studies have led to the general consensus that enhancers and promoters physically interact when the enhancer or gene is active. However, the experimental evidence that underlies this conclusion is not fully consistent and contains some conflicting results. In some cases, distal enhancers are always in proximity with their target gene promoters, irrespective whether they are active or not (termed pre-formed or permissive contacts)[3–6]. In other examples, enhancers only interact with promoters if they are active (instructive contacts)[6–9]. In addition, at some loci, the physical distance between enhancers and promoters would even increase (slightly) when enhancers are active[10,11]. Extrapolating general principles on enhancer function from such a multitude of scenarios derived from different tissues, developmental stages, and species remains a challenge. If and how much enhancer–promoter interactions change in response to enhancer activation are less well supported by data than one would assume. Technically, it is hard to determine enhancer–promoter contacts owing to limitations of the 3C-based technology. For example, Hi-C experiments often lack the resolution to investigate chromatin contacts of enhancers, which are typically a few hundred base-pairs long. In addition, most high-resolution experiments have been performed only on frequently used cell types that rarely include those relevant for well-characterized developmental enhancers that for many years have served as work-horse for understanding gene regulatory elements.

To overcome these challenges, Pollex et al.[1] and Chen et al.[2] focus on large, well-defined sets of developmental enhancers in *Drosophila* and mouse embryos to investigate how their chromatin interactions differ in various tissues or stages of embryonic development (Fig. 1). To achieve this, both groups use capture-C, which provides chromatin maps at higher resolution than Hi-C, and they rigorously validate their findings by applying several orthogonal methods such as DNA–FISH, enhancer reporters, and CRISPR-knockouts.

Pollex et al.[1] focus on a set of 600 hand-selected enhancers and promoters with known activity patterns in the myogenic or neuronal lineages during *Drosophila* embryogenesis. They perform capture-C, chromatin immunoprecipitation with sequencing (ChIP–seq) and DNase-sequencing analysis in FACS-purified nuclei of multipotent, myogenic or neurogenic cells during three embryonic time points that reflect distinct stages of cell fate specification and differentiation. The authors[1] find that at early stages, during the speciation of cell fate, enhancer–promoter interactions are overall fewer and fairly similar, even between myo-/neuroblasts that already committed to their lineage. Consistent with earlier findings[3], these interactions are predominantly pre-formed. By contrast, at later stages during neuron and myocyte differentiation, many new enhancer–promoter connections are formed and correlate with a specific enhancer being active at this stage. In other words, enhancer–promoter interactions are more permissive at earlier stages, and more instructive at later stages. The temporal aspect of these findings is a particular strength of this study[1], as it highlights different predominant patterns of enhancer–promoter interactions in different phases of development. That gene regulatory control might follow a different logic in more differentiated tissues or cells, as compared to early embryonic stages or cell culture models, is often overlooked, with olfactory neurons being a well-characterized, notable exception[12].

By specifically chasing functional interactions, several interesting further observations provide nuance to the broader conclusions of the study[1]. For example, a prominent interaction between the *Toll-7* promoter and a putative enhancer turns out to be a cell type-specific interaction with the promoter of a long noncoding RNA, which the authors then demonstrate to regulate *Toll-7* expression with a series of mutant fly lines. In another series of validation experiments, enhancer–reporter assays for regions that interact with the *Oli* gene in neuronal cell types highlight that not all *Oli*-interacting regions act as textbook



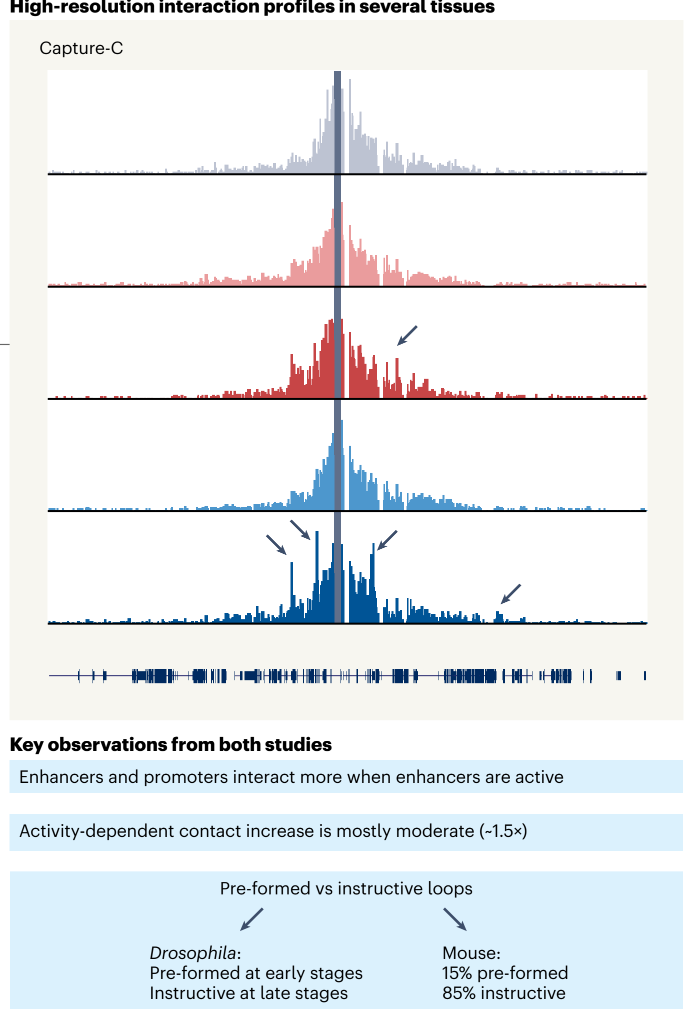


Fig. 1 | High-resolution mapping of enhancer chromatin interactions in vivo reveals principles of activity-dependent three-dimensional contacts. Combination of selected embryonic cell lineages and tissues (top left) with a tailored set of *cis*-regulatory elements as capture-C enrichment baits (bottom left) allows systematic detection of cell-type specific chromatin interactions (arrows, top right). Some key observations drawn from both studies are summarized (bottom right).

enhancers. Both observations suggest that capture-C assays can identify functionally important regions even though these might follow a different logic than 'canonical' enhancers, which paves the way for interesting new research directions.

In the other study, Chen et al.[2] use the VISTA enhancer database[13], built over more than a decade to identify tissue-specific interaction patterns for approximately 1,000 mouse enhancers, which has been useful to estimate the abundance of tissue-specific activity of numerous mouse and human enhancer elements and interpreting human disease-related variants. With a capture Hi-C assay using known enhancers as baits, the authors[2] profiled the chromatin interaction partners in 10 different tissues of mid-gestation mouse embryos. Similar to the *Drosophila* study[1], this systematic approach provides data for a more comprehensive view and generalized conclusions on enhancer–promoter looping during developmental gene activation, confirming that the nearest promoters are frequently bypassed and that active enhancers contact each other in clusters. Two results stand out. First, most enhancers profiled (>85%) form tissue-specific (that is, instructive) chromatin loops with their target promoters, in those tissues where they are active. Only the minority (13.3%) of enhancer–promoter interactions form stably across tissues, which can mostly be explained by nearby CTCF sites as likely drivers for this interaction. However, irrespective of interaction type, enhancer–promoter contacts are increased in those tissues where the enhancer is active. A second important conclusion, strengthened by the breadth of the database used in the study, is that the measurable increase of enhancer–promoter interaction between active and inactive states is rarely dramatic (although in some cases it is). In fact, the average increase between 'active/inactive' interaction is only around 1.5-fold. For a subset of enhancer–promoter interactions, the authors[2] confirm the increase with super-resolution three-dimensional (3D) DNA fluorescence in situ hybridization (DNA–FISH), in which they find an approximately 1.5-times higher colocalization between enhancer and promoter regions in tissues where the enhancer or gene is active.

Albeit modest, the increase can have a notable effect on the transcription of target genes, consistent with the results of a recent study on an engineered model locus in mouse embryonic stem cells that showed a positive, but non-linear relationship between enhancer–promoter proximity and transcriptional output[14].

Building on a well-curated set of enhancers, capture-C in suitable in vivo samples and extensive functional validation, these studies provide depth and context to previous literature reports. Although single-locus studies highlighted and dissected different aspects of enhancer–promoter interactions, general conclusions were difficult to draw because of peculiarities of individual loci, model organisms or regulatory scenario. Pollex et al.[1] and Chen et al.[2] show that enhancer–promoter interaction will increase in tissues where the enhancer is active and already a modest increase seems to be sufficient for the enhancer to be functional. At the same time, these results widen our view, leaving a series of unanswered questions. How do multiple enhancers interact? How is a modest increase in interaction transformed to higher transcriptional response? What role do insulator proteins have? Why are some promoters activated by enhancers, whereas others are skipped? To address these important questions, single-locus studies will be needed to dissect mechanisms. However, they should go hand-in-hand with systematic studies such as these to test whether models developed at exemplary loci prove to be the exception or the rule.

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Competing interests

The author declares no competing interests.