TRANSCRIPTIONAL REGULATION

Visualizing long-range enhancer–promoter interaction

A new study uses multicolor live imaging to simultaneously visualize enhancer–promoter interaction and transcription in Drosophila embryos.

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Transcriptional enhancers are short DNA fragments that remotely control gene expression, being able to drive transcription from promoters located tens of thousands of base pairs away. Several decades have passed since their initial discovery, and we are just beginning to unravel how enhancers and promoters interact in vivo. A long-standing question in the field remains: how does the physical interaction between enhancers and promoters impact gene expression? In this issue, Chen et al.1 describe an imaging approach to directly monitor long-range interactions between promoters and distal enhancers and their consequences on transcriptional output.

Transcriptional enhancers in multicellular eukaryotes greatly outnumber genes. In the case of humans, estimates for the total number of enhancers exceed a million elements2,3. This number stands in stark contrast to the estimated ~20,000 genes. Thus, each promoter is, on average, under the control of more than ten regulatory elements. Furthermore, the distances between enhancers and promoters vary over a wide range—with some separated by over a million base pairs4. However, the mechanisms and functional significance of enhancer–promoter interaction have remained enduring questions.

One shortcoming in the field has been that most measurements concerning enhancer–promoter communication are based on imaging or sequencing studies from fixed samples. Such assays do not often capture the temporal dynamics of transcriptional interactions, leading to many outstanding questions. What kind of physical interactions are required to drive transcription? How close must the enhancer be to the promoter to drive transcription? How frequent or stable are enhancer–promoter interactions? How do these interactions shape animal development? With recent reports hinting that transcription is dynamic and even stochastic, live imaging studies will necessarily have a pivotal role.

Enhancer–promoter interaction goes live

Chen et al.1 imaged the positions of enhancers relative to eve promoters within live Drosophila embryos. eve is a well-characterized developmental locus expressed in seven stripes along the anterior-to-posterior axis of early Drosophila embryos, patterning corresponding body segments. The authors first tagged the native eve mRNA with binding sites for fluorescent viral coat proteins (from MS2)4,5, providing a direct readout of transcription from the endogenous locus. Enhancers driving stripes of eve expression are located proximal to the native promoter, which allowed the authors to monitor the location of eve enhancers using MS2 coat protein fluorescence as a proxy. The authors additionally inserted a remote reporter construct 142 kb away. It contained ParB binding sites to mark its location7,8 and a reporter mRNA tagged with PP7 stem loops under the control of an eve promoter. Collectively, this three-color setup allowed the authors to measure the physical distance between the native eve enhancers and the distal reporter construct, providing a direct readout of long-range transcriptional regulation (Fig. 1).

A hallmark of early Drosophila embryos is their rapid nuclear divisions, occurring with timing on the order of 30 min or less. These rapid cell cycles potentially leave insufficient time for long-range interactions to form. To facilitate the formation of stable looping interactions, the authors took advantage of a homie insulator element present in the native eve locus9 and added a corresponding insulator to the distal reporter construct. With pairing insulator elements, the distal reporter became transcriptionally active, even with the rapid nuclear divisions.

The authors observed that the distances between enhancers and promoters were shorter when the reporter was transcriptionally active. In fact, immediately before transcriptional activation, the distance between the
enhancer and promoter decreased by around twofold. Conversely, when the distance between the enhancer and promoter increased, transcription stopped. These findings suggest that sustained physical proximity of regulatory elements is required for transcription.

Further characterizing the dynamics of long-range gene regulation, the authors found that a model with three states fit the distribution of distances between regulatory elements. These states represented three functional conformations, concerning physical distance (distal versus proximal) and transcriptional state (on versus off): distal off, proximal off and proximal on. Deletion of individual components in the reporter construct supported this interpretation. Tracking the populations of each state over time highlighted the striking observation that sustained proximity is required to transition into the on state.

The future of live-imaging transcription

Chen et al. demonstrated the power of using in vivo imaging to confirm that enhancer–promoter interactions are required for sustained transcription. These results highlight the relationship between DNA topological association and physical proximity. That is, elements that strongly influence the conformation of chromosomes directly alter gene regulation. The *home* insulator element chosen by the authors is a particularly strong example of this kind of element, as it was able to hijack transcription from the native eve promoter to the distal reporter construct, leading to competition between promoters resulting in developmental defects. Furthermore, while many regions in the genome are bound by similar insulator or architectural proteins (i.e., CTCF and BEAF32), it is not clear how many of these regions are functional or how many elements have ‘homing’ activity. Future work could address the generalities of such insulator function and its dynamics, leading to a deeper understanding of transcriptional regulation.

These findings are consistent with the suggestion that chromosomal boundaries are rather stable, based on the similarity of boundaries across cell types from genomic data. However, the dynamics of the loop structures that form such insulated neighborhoods and the enhancer–promoter interactions within these regions are not yet fully understood. Furthermore, in the *Drosophila* genome, there is evidence for stable, long-range interactions between loci, consistent with the findings of Chen et al. In fact, the majority of enhancer interactions show no evidence of dynamic changes across development. The experimental approaches used by Chen et al. could be applied to study the dynamics of such neighborhoods of gene expression and enhancer–promoter interactions.

Intriguingly, the finding that stable physical proximity of regulatory elements is required for transcription seems to be at odds with recent work demonstrating that active transcription sites are highly dynamic and can diffuse more freely. These differences may be the result of the insulator elements inducing a very stable chromatin conformation, working with timing on the order of tens of minutes. In contrast, recent studies focused on transcriptional dynamics occurring in time frames from milliseconds to minutes. Resolving the apparent inconsistencies between the stable, long-range chromatin topologies found by Chen et al. and the dynamic local transcriptional environments may be the key to understanding the mechanisms of transcriptional regulation during development. It is intriguing that many of these results are converging on similarly sized transcriptional ‘hubs’, which may form transiently on the stable chromatin scaffolds formed by insulator proteins. Ultimately, continued work to connect genomics to these in vivo measurements promises to lead to a better understanding of how the molecular interactions within the nucleus lead to a fully functional organism.

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Competing interests

The authors declare no competing interests.