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Genome organization controls transcriptional dynamics during development

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Abstract

Past studies offer contradictory claims for the role of genome organization in the regulation of gene activity. Here, we show through high-resolution chromosome conformation analysis that the *Drosophila* genome is organized by two independent classes of regulatory sequences, tethering elements and insulators. Quantitative live imaging and targeted genome editing demonstrate that this two-tiered organization is critical for the precise temporal dynamics of Hox gene transcription during development. Tethering elements mediate long-range enhancer-promoter interactions and foster fast activation kinetics. Conversely, the boundaries of topologically associating domains (TADs) prevent spurious interactions with enhancers and silencers located in neighboring TADs. These two levels of genome organization operate independently of one another to ensure precision of transcriptional dynamics and the reliability of complex patterning processes.

Genome organization is emerging as a potentially important facet of gene regulation (1–5). Because transcriptional enhancers often reside far from their target promoters, chromatin folding may guide the timely and specific establishment of regulatory interactions (1, 3, 4, 6–10). Although long-range enhancer-promoter contacts are prevalent, it remains unclear whether they actually determine transcriptional activity (9, 11). Boundary elements partition chromosomes into topologically associating domains (TADs) (7, 12), whose importance for gene regulation remains controversial (8, 13–16). There is also an unresolved

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SUPPLEMENTARY MATERIALS
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dichotomy between elements that promote and prevent enhancer-promoter interactions, because CTCF binding sites have been implicated in both (7, 9, 17). We show here that distinct classes of regulatory elements mediate these opposing functions genome-wide: Dedicated tethering elements foster appropriate enhancer-promoter interactions and are key to fast activation kinetics, whereas insulators prevent spurious interactions and regulatory interference between neighboring TADs.

We characterized genome organization at single-nucleosome resolution in developing Drosophila embryos using Micro-C (18). We focused on the critical ~*60*-min period preceding gastrulation, when the fate map of the embryo is established by localized transcription of a cascade of patterning genes, culminating with the Hox genes that specify segment identity. Analysis of the *Antennapedia* gene complex (ANT-C), one of two Hox gene clusters and an archetype of regulatory precision, reveals an intricate hierarchical organization. Insulators partition the locus into a series of TADs, whereas tethering elements mediate specific intra-TAD focal contacts between promoters of *Scr* and *Antp* and their distal regulatory regions (Fig. 1A and fig. S1).

The entire genome is similarly organized by 2034 insulators and 620 tethering elements. Insulators and tethers display notably little physical overlap (Fig. 1B) and have sharply contrasting chromatin signatures (Fig. 1C; fig. S2, A to C; and table S1). Insulators are characterized by H3 lysine 4 trimethylation (H3K4me3) and the binding of canonical insulator proteins (CTCF, CP190), whereas tethers are distinguished by H3K4 monomethylation (H3K4me1) and the binding of pioneer factors Trithorax-like (Trl), grainyhead (grh), and zelda (zld; fig. S2, A and B). There are 103 focal contacts (33%) that connect promoters of protein-coding genes to “orphan” intergenic sequences, which we term distal tethering elements (DTEs; Fig. 1D); others connect different genes together. These contacts typically span tens of kilobases (mean 43.5 kb; Fig. 1D and table S2) and are observed at many critical developmental loci, including *vestigial* and *cut* (fig. S3). Because DTEs generally display no enhancer activity in the early embryo (Fig. 1E and fig. S2D), we hypothesized that they might be organizational elements dedicated to fostering long-range enhancer-promoter interactions. In contrast to enhancers, DTEs retain an “open” chromatin conformation throughout embryogenesis (fig. S2E), consistent with evidence that focal contacts are stable across cell types (19) and developmental stages (11). To explore their potential roles in transcriptional regulation, we systematically disrupted tethers and insulators throughout the ANT-C (tables S3 and S4) and leveraged quantitative live-imaging methods to measure changes in the transcriptional dynamics of Hox genes in developing embryos (Fig. 1F).

The *Sex combs reduced* (*Scr*) gene, contained within a 90-kb TAD, is regulated by an early embryonic enhancer (*Scr* EE) located *35* kb upstream of the promoter [figs. S4 and S5; (20)]. This enhancer bypasses an intervening TAD that contains *ftz*—a highly expressed pair-rule gene—to selectively regulate *Scr* transcription. A DTE situated 6 kb upstream of the enhancer anchors a focal contact with a promoter-proximal tether (Fig. 2A). These tethering elements correspond to sequences previously shown by reporter assays to modulate enhancer-promoter selectivity (21, 22). The DTE lacks any intrinsic enhancer activity (fig. S4), suggesting a specific role in fostering long-range enhancer-promoter interactions.

A targeted deletion of the DTE completely abolishes this focal contact and diminishes interactions between the EE enhancer and the *Scr* promoter (Fig. 2A and figs. S6 and S7). Single-cell transcription measurements in living embryos reveal a marked delay in the dynamics of *Scr* activation across the cells of the prospective stripe (Fig. 2B). Transcription levels in nuclei that become active appear unaffected, and the mutant allele ultimately reaches a regime of activity indistinguishable from that of the wild type. Overall, the mutant allele is active in the appropriate spatial domain, but its transcriptional output is substantially reduced owing to the delayed onset of expression (Fig. 2B and fig. S6A). Deletion of the EE enhancer reduces *Scr* transcription but does not disrupt the focal contact—it may even be somewhat strengthened (Fig. 2A and fig. S8). These observations suggest that promoter-DTE focal contacts are autonomous features of the regulatory genome. Disruptions of focal contacts have strictly gene-specific effects: Deletion of the *Scr* DTE has no impact on the structure or transcription of the neighboring *Dfd* locus (fig. S6, C to E).

Similarly, the *Antennapedia* (*Antp*) P1 early enhancer is associated with a DTE directly adjacent to it, which forms a focal interaction with a tethering element near the P1 promoter, 38 kb away. Upon deletion of the DTE, the focal interaction is lost, and enhancer-promoter interactions are disrupted (figs. S6 and S7). *Antp* activation is substantially delayed but transcription levels in active nuclei are normal, and transcription appears to fully recover after this initial lag (fig. S9).

These observations show that DTEs specifically determine the dynamics of transcriptional activation in development. This temporal precision may be critical for the programming of cellular identities within stringent developmental windows. We propose that tethering elements foster physical interactions between promoters and remote enhancers to prime genes for rapid activation; they may also modulate other aspects of enhancer-promoter communication through interactions with core transcription complexes.

In addition to fostering preferential associations with target promoters, DTEs also suppress “backward” interactions of associated enhancers with distal regions of their TADs (Fig. 2A and fig. S7). Both effects probably synergize to increase the specificity of enhancer-promoter communication. Although DTE deletions have a strong impact on local genome organization, they have little effect on the overall structure of TADs (Fig. 2A and fig. S7), suggesting that insulators and tethering elements operate largely independently of one another. To better understand the relationship between long-range enhancer-promoter interactions and TAD structures, we systematically disrupted each of the TAD boundaries across the *Dfd-Scr-Antp* interval (tables S3 and S4).

Deletion of the *Dfd* 3′ insulator causes a wholesale fusion of the *Dfd* TAD with the adjacent *miR-10* TAD and reduces transcription of the *Dfd* gene (Fig. 3A and figs. S10 to S12). Notably, it does not appear to weaken interactions between the *Dfd* promoter and enhancer, suggesting that TAD boundaries play no role in fostering appropriate regulatory interactions. Rather, the 3′ insulator specifically prevents inappropriate contacts with the *miR-10* regulatory region.

Similarly, individual deletions of the boundaries of the *ftz* TAD, which is nested within the *Scr* locus, cause fusions with either side of the *Scr* TAD. The remaining insulator continues to enforce a robust boundary (Fig. 3B and figs. S10 to S12). *Scr* transcription is markedly reduced in both cases, though the deletion of *SF1* has a substantially more severe impact than *SF2* (Fig. 3B and fig. S10). Neither deletion disrupts the promoter-DTE interaction (fig. S11), suggesting that TAD boundaries are not required for the establishment or maintenance of long-range focal contacts. This supports the view that tethers and boundaries constitute independent levels of organization, as suggested by our genome-wide analysis.

The disruption of *Scr* TAD boundaries is also consistent with this model. Deletion of the *Scr* 3′ insulator is recessive lethal, probably because of the loss of essential 7SL genes, and could not be analyzed by Micro-C. But a targeted deletion of the *Antp* 3′ intronic insulator is viable and causes a partial fusion of the *Scr* and *Antp* P2 TADs (figs. S10 to S12). The persistence of a residual boundary can be explained by the presence of a secondary insulator located ~4 kb away. Deletion of either *Scr* TAD boundary severely reduces *Scr* transcription (Fig. 3C and figs. S10 and S11). Notably, disruption of the *Scr-Antp* boundary does not weaken the interaction of the DTE with the *Scr* promoter (fig. S11), suggesting that reduced *Scr* expression is not due to diminished enhancer-promoter interactions. This partial fusion of the *Scr* and *Antp* P2 TADs has, at most, only a marginal impact on *Antp* transcription (Fig. 3D and figs. S10 and S11), revealing that boundary deletions can have sharply asymmetric regulatory effects on flanking TADs.

Because TAD boundary deletions do not alter appropriate enhancer-promoter interactions, we sought an alternative explanation for reduced *Scr* transcription arising from disruptions of the *ftz* TAD. *SF1* removal exposes the *Scr* promoter to interactions with the *ftz* regulatory region (Fig. 3E and fig. S11), which may thus directly interfere with *Scr* transcription. By contrast, *SF2* removal allows *ftz* regulatory sequences to interact with the EE enhancer (fig. S11), but not directly with the *Scr* promoter (Fig. 3E and fig. S11), which may explain its more subtle transcriptional impact. In the absence of *SF1*, the severely narrowed *Scr* domain and distinctive ectopic stripes suggest both activation and silencing by *ftz* enhancers (fig. S13). A prime suspect for this altered expression pattern is the AE1 enhancer, which binds both activators and the Hairy repressor (fig. S13). Indeed, the AE1 element functions as a potent silencer within the *Scr* expression domain (Fig. 3F and fig. S10), and *Scr* transcription faithfully mirrors AE1 activity upon *SF1* removal (fig. S13). We conclude that the primary function of insulators is to prevent regulatory interference between TADs, and this can explain even surprising quantitative differences in the transcriptional effects of boundary deletions.

To assess the functional importance of tethering elements and insulators, we analyzed the number of teeth on the sex combs of adult males, a quantitative phenotype under sexual selection governed by *Scr* expression. All relevant deletions reduce the average number of teeth, and the magnitude of the transcriptional defects is highly predictive of the severity of the morphological phenotypes (Fig. 4, A and B, and fig. S14). These observations demonstrate the importance of genome structure for the control of transcriptional dynamics and the precision of developmental patterning.

Taken together, our observations support a general model in which genome organization canalizes regulatory interactions through two classes of organizing elements with diametrically opposing functions. A dedicated class of tethering elements, often physically distinct from enhancers, foster enhancer-promoter interactions and are key to fast transcriptional activation kinetics during development (Fig. 4C). We anticipate that similar mechanisms will prove to be an important property of vertebrate genomes, where large distances often separate genes from their regulatory sequences (9, 23, 24). By contrast, TAD boundaries have a pervasive role in enforcing regulatory specificity by preventing interference between neighboring TADs (Fig. 4C).

Although prior studies have emphasized the spatial regulation of gene expression, temporal dynamics have proven far more elusive. Quantitative measurements in live embryos revealed clear delays in the onset of transcription upon deletion of tethering elements. The Trl protein, which binds most of these sequences, has been proposed to act as a DNA looping factor (25, 26). We suggest that tethering elements "jump-start" expression by establishing enhancer-promoter loops before activation, though it is likely that they also serve a broader function. Indeed, it is intriguing that the *Scr* DTE co-incides with a classical Polycomb response element (27). This is consistent with a possible role for Polycomb repressive complex 1 (PRC1) components in the establishment of enhancer-promoter loops (28) and suggests that focal contacts constitute a versatile topological infrastructure used by a variety of regulatory mechanisms. Our study shows that genome organization shapes transcription dynamics through two complementary mechanisms: Tethering elements foster appropriate enhancer-promoter interactions, whereas TAD boundaries prevent inappropriate associations.

Supplementary Material

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Data and materials availability:

All Micro-C sequencing data are available through GEO accession number GSE171396. All imaging data are freely available upon request. All materials used in the analysis are available upon request, and custom data analysis code is available through GitHub (https://github.com/phil-batut/transcription_imaging.git) and Zenodo (29).

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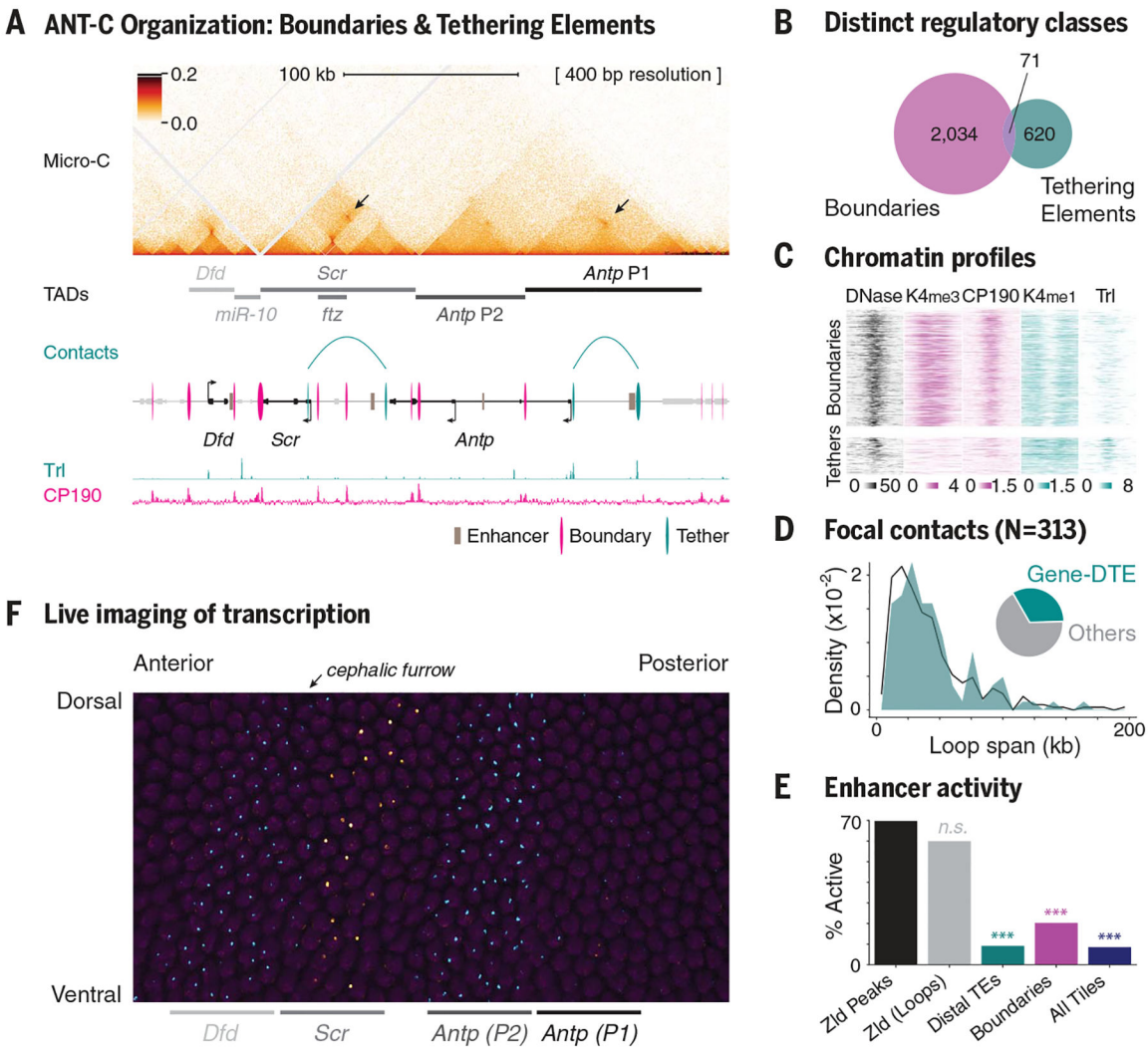

Fig. 1. Hierarchical genome organization: Boundaries and focal contacts.

(**A**) ANT-C organization (*Dfd-Antp* interval). The following are shown from top to bottom: Micro-C contact map showing TADs and focal contacts (arrows); Hox genes (black); other genes (gray); regulatory elements; and chromatin immunoprecipitation (ChIP) data for Trl and CP190. (**B**) Tethers and boundaries are physically distinct. (**C**) Epigenetic signatures of tethers and boundaries. DNase, deoxyribonuclease I. (**D**) Fraction of contacts connecting gene promoters to "orphan" DTEs and a histogram of loop spans (black, all loops). (**E**) Enhancer activity, by functional class (***$p < 10^{-7}$ versus Zld peaks, Bonferroni-corrected chi-square test; n.s., not significant). (**F**) Image of a live embryo showing transcription of *Dfd, Antp* (cyan), and *Scr* (yellow, image enhanced), with nuclei in purple.

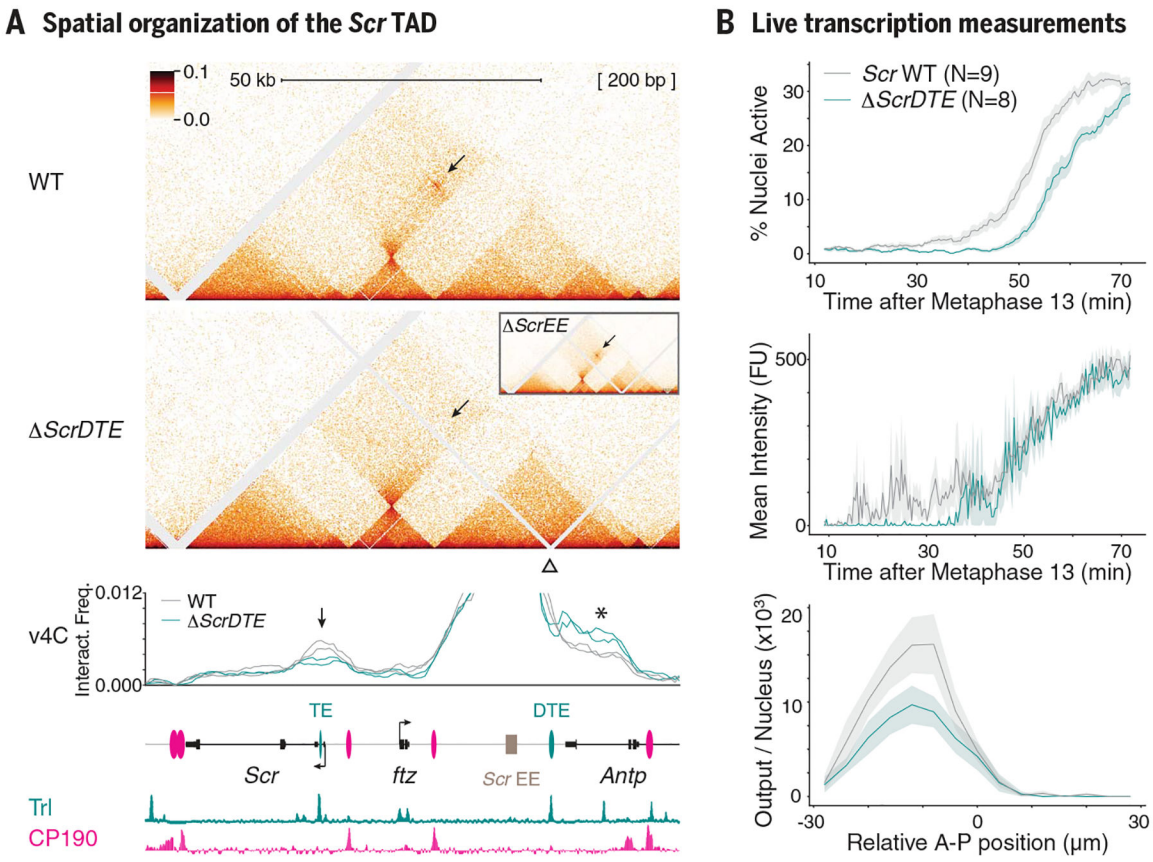

Fig. 2. Tethering elements foster enhancer-promoter interactions and control activation kinetics.

(**A**) Micro-C for *Scr* DTE mutant embryos. (The triangle indicates the location of the deletion.) Virtual 4C (v4C) shows decreased interactions of the EE enhancer with the promoter upon DTE deletion (arrow) and increased interactions with regions beyond the DTE (asterisk). The focal contact persists in **Δ*ScrEE*** embryos (inset). (**B**) Live measurements of endogenous *Scr* transcription show delayed activation in **Δ*ScrDTE*** embryos. A-P, anterior-posterior; FU, fluorescence units; N, number of embryos; shading, ±SEM).

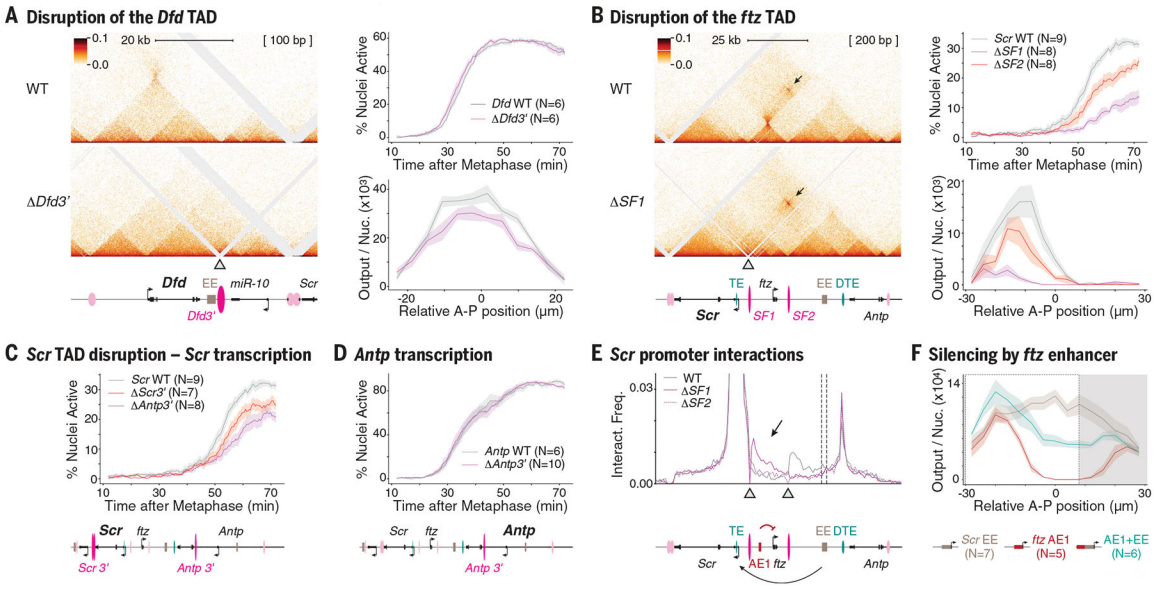

Fig. 3. Insulators prevent regulatory interference and promote transcriptional precision.

(**A**) Micro-C and *Dfd* transcription measurements for **Δ***Dfd3′* insulator mutant embryos. The triangle indicates the location of the deletion. (**B**) Micro-C and *Scr* transcription measurements for **Δ***SF1* (and **Δ***SF2*) embryos. The focal contact persists (arrows). (**C**) *Scr* transcription in **Δ***Scr3′* and **Δ***Antp3′* embryos. (**D**) Antp transcription in **Δ***Antp3′* embryos. (**E**) Interaction landscape of the *Scr* promoter upon disruption of the *ftz* TAD (see Micro-C above). (**F**) Reporter assay showing silencing by the AE1 enhancer within the *Scr* expression domain (dashed box). In all panels, shading indicates ±SEM.

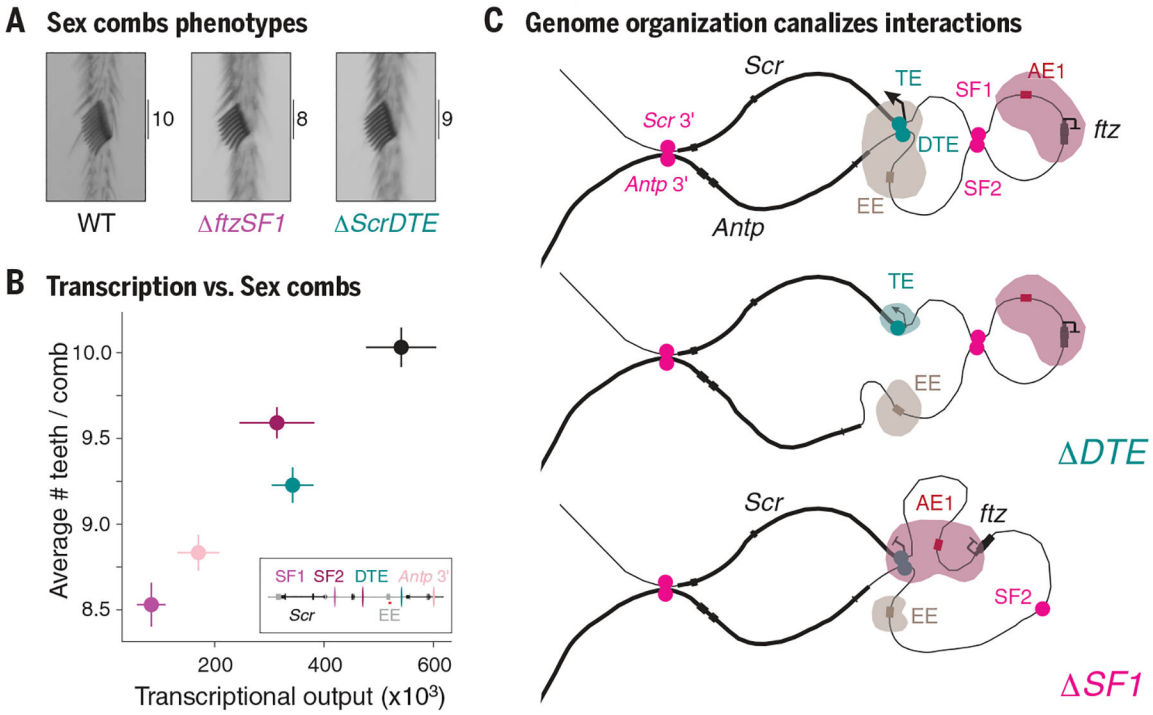

Fig. 4. Genome organization controls transcriptional dynamics and developmental patterning.

(**A**) Representative images of sex combs from adult males. (Numbers indicate tooth counts.) (**B**) Correlation of transcriptional output and tooth count (inset, locus map; red bar, sex comb enhancer; error bars, ±SEM). (**C**) Organization of the *Scr* locus: Tethers foster specific enhancer-promoter interactions, whereas boundaries prevent regulatory interference between TADs.