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Enhancer-promoter communication: hubs or loops?

Bomyi Lim[1] and Michael S Levine[2,3]

There has been a sea change in our view of transcription regulation during the past decade (Fukaya *et al.*, 2016, Lim *et al.*, 2018, Hnisz *et al.*, 2017 [3], Liu *et al.*, 2018 [4], Kato *et al.*, 2012). Classical models of enhancer-promoter interactions and the stepwise assembly of individual RNA Polymerase II (Pol II) complexes have given way to the realization that active transcription foci contain clusters—hubs—of transcriptional activators and Pol II. Here we summarize recent findings pointing to the occurrence of transcription hubs and the implications of such hubs on the regulation of gene activity.

Addresses
[1] Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA 19104, United States
[2] Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ 08544, United States
[3] Department of Molecular Biology, Princeton University, Princeton, NJ 08544, United States

Corresponding author: Levine, Michael S (msl2@princeton.edu)

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Transcription hubs

The hub hypothesis incorporates three key observations that challenge classical views of enhancer-promoter communication [1–5]. First, transcription is not continuous but instead occurs in bursts [6]. Second, a single enhancer can co-activate the transcription of linked reporter genes [1]. And third, large distances separate enhancers and promoters during activation [7,8••].

Concurrent with these observations is emerging evidence for clusters or condensates of transcriptional regulators at active loci [5,9–11]. The classical view is that individual sequence-specific transcription factors (TFs) bind to cognate binding sites contained within their target enhancers and then persist for long periods due to slow off rates [12]. Instead, the emerging view is that TFs dynamically bind and dissociate from their target sites, and occupancy is sustained by high local concentrations—clusters or condensates—of TFs [5,13].

Several TFs have been shown to form clusters at active transcription loci in living *Drosophila* embryos, including Bicoid, Zelda, and Ultrabithorax [14,15,16•,17,18•]. These studies suggest that local enrichment of TFs at transcription foci facilitates occupancy of low-affinity binding sites within an enhancer. TF clusters have been observed in other systems as well, including Mig1 in yeast, Oct4 and Sox2 in embryonic stem cells, EWS/FLI1 in Ewing's sarcoma cells, and YAP in MCF-10A cells [9,19–21]. The activation domains of TFs often contain intrinsically disordered regions (IDRs) that can drive phase separation processes *in vitro* and *in vivo* [9,22]. Interestingly, these condensates have been shown to associate with subunits of the Mediator and Pol II complexes, suggesting a mechanism for enhancer-promoter interactions through phase separation [5,10,22].

Pol II clusters

Similarly, with few exceptions, promoters were previously viewed as 'naked' before the onset of transcription [23]. TFs were thought to trigger the stepwise assembly of the pre-initiation complex (PIC), leading to the recruitment, activation, and release of individual Pol II complexes [24]. More recent studies document the widespread occurrence of paused Pol II [25,26]. It was originally envisioned that a single Pol II complex stably arrests (~10 min or longer half-life) in promoter proximal regions, ~30–50 bp downstream of the transcription start site [25]. Instead, it appears that Pol II achieves 'occupancy' of pause sites through the formation of transient clusters (~10–60 s half-life). The more stable of these clusters (>60 s half-life) co-localize with Mediators at sites of active transcription [11,27]. In addition, condensates containing Mediator and its co-factor BRD4 have been observed at superenhancers, implying the formation of multivalent condensates that are accessible by both enhancers and promoters [10].

In the past few years, there have been many efforts to identify and characterize phase separated condensates in transcriptional regulation. Recent studies suggest that TF condensates localized at enhancers recruit Pol II and Mediators to form an activation hub at target promoters [9,22,28•]. Expanding the number of copies of HOXD13 IDRs was recently shown to induce phase separated condensates with BRD4. Interestingly, adding alanine repeats to the IDRs led to the exclusion of Mediator and Pol II, resulting in reduced activation of HOXD13-target genes [29].

Phase separation might play a role in gene repression as well, as suggested by the analysis of HP1a/α in heterochromatin [30,31]. Moreover, mutations in the CBX2 subunit of the Polycomb PRC1 complex disrupt formation of phase separated condensates and cause developmental defects in mice [32•]. The TLE/Groucho corepressor forms oligomers and interacts with HDACs, a process that is evocative of phase separation [33].

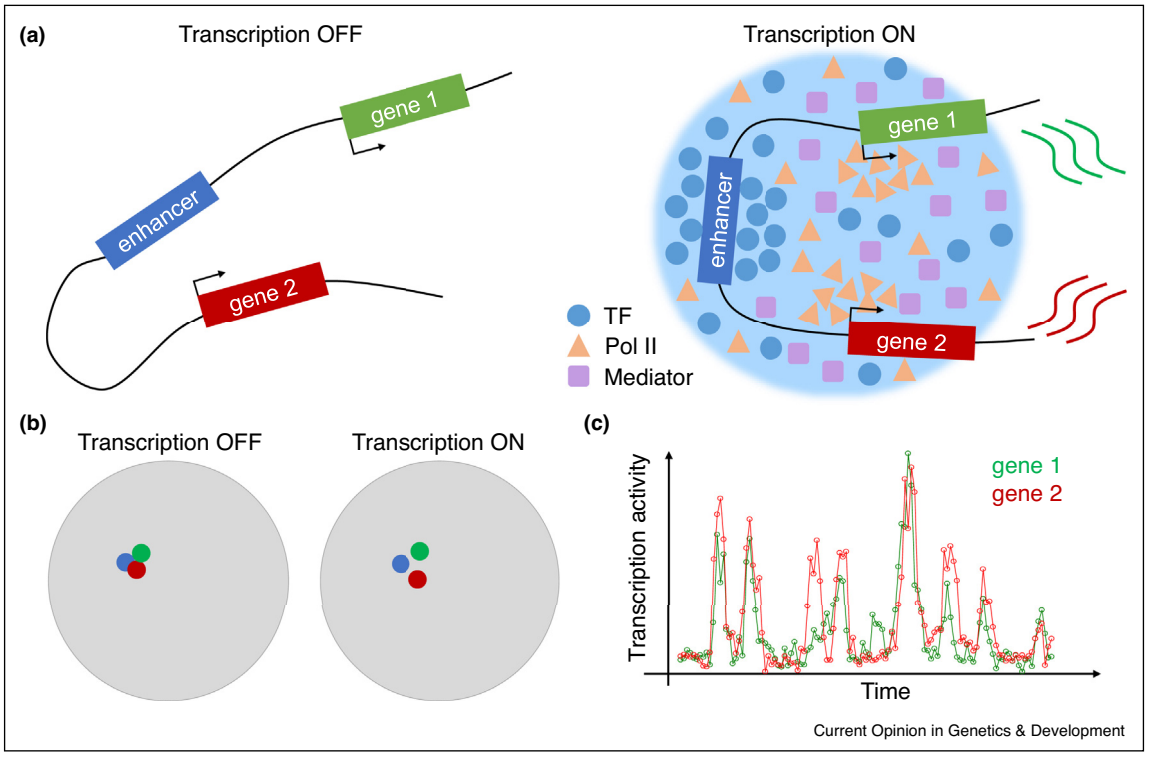
Despite increasing reports of TFs + Pol II condensates at active transcription foci, there are many limitations in our understanding of how clusters or condensates of TFs at enhancers interact with clusters or condensates of Pol II at promoters to form transcription hubs regulating the expression of associated genes. Quantitative studies will be required to confirm whether the observed TFs and Pol II clusters demonstrate properties of liquid–liquid phase separation [34]. But once formed, hubs—or activation condensates (TFs + Pol II)—help explain a number of perplexing observations that are seemingly incompatible with classical models of enhancer-promoter communication (Figure 1).

Transcriptional Bursting

Discontinuous, episodic bursts of transcriptional activity have been observed in a variety of systems and cell types [35–39]. Yet, we currently do not understand the mechanisms responsible for bursting. It was recently suggested that they might arise, in part, from the recycling of elongating Pol II within a gene 'puff' or loop [40]. Nonetheless, bursts are certainly compatible with the emerging view that active promoters contain clusters or condensates of Pol II. It is easy to imagine how induction of transcription hubs could lead to the rapid and sequential release of several Pol II complexes to obtain a burst of transcription.

Indeed, it was shown that Pol II clustering occurs before pause release [27], and could be inhibited by specific small drug molecules. Bursting resumed upon removal of the drugs, suggesting that Pol II clusters at promoters can regulate the size and frequency of bursts [41]. Recent studies are also consistent with the idea that the rate of Pol II pause release is a key determinant of transcriptional bursting [42]. The carboxy-terminal domain (CTD) of

Figure 1



(a) Formation of transcription hub as a possible mechanism for gene regulation. When transcription is active, TFs, Pol II, Mediators and other transcription machineries can form multivalent phase-separated condensates (or clusters) that facilitate enhancer-promoter interactions for transcriptional initiation.
(b) A schematic of the visualization of enhancer (blue), gene 1 (green), and gene 2 (red) loci shown in (a) when transcription is OFF and ON. The distance between enhancer and the target promoter is not as close as expected (200–400 nm), possibly due to large size of higher order associations of transcription complexes.
(c) A representative transcriptional trajectory from two linked reporter genes regulated by a single enhancer. The occurrence of coordinate busting can be explained by the transcription hub model. Data are obtained from Ref. [1].

Pol II mediates phase separation, and phosphorylation of CTD releases Pol II from transcription hubs (TFs + Pol II) to initiate transcription elongation [43]. Moreover, it was recently shown that CTD length influences the size and frequency of transcriptional bursting [44]. While the transcription hub model might explain some properties of bursting, further studies are needed to fully understand this process.

Coactivation

The analysis of linked reporter genes led to the surprising observation that a shared enhancer can mediate coordinated bursting of MS2-tagged and PP7-tagged reporter genes (Figure 1c) [1]. This observation contrasts with the classical enhancer-promoter looping models, which predicted sequential activation of linked genes, one at a time [45]. Coordinate bursts are compatible with the hub hypothesis. According to this view, both target promoters share a common pool (cluster or condensate) of TFs and Pol II. Induction of the hub could lead to the release of individual Pol II complexes onto both promoters, leading to coordinate bursting.

Remarkably, co-activation is also observed for reporter genes located on different homologous chromosomes (transvection) [2]. In the presence of closely linked insulator DNAs, an enhancer on one allele was able to activate both a *cis*-linked PP7 reporter gene and a *trans*-linked MS2 reporter gene located on the other homologue. Co-activation was detected only when the two alleles were in close physical proximity [2,7]. This observation suggests that transcription hubs can span different chromosomes, if brought into proximity. To date, coordinate bursts have only been observed for synthetic reporter genes, and it remains to be seen if linked genes within endogenous loci display similar coordinate behaviors. Although visualization of linked genes within endogenous loci has not yet been done, it was recently shown that endogenous α-globin enhancers can interact with multiple promoters within the same Topologically Associating Domain (TAD), consistent with the hub model [46].

Large distances

Classical models of enhancer-promoter looping envisioned intimate contact of distal enhancers with their target promoters [45]. However, the occurrence of clusters or condensates of TFs and Pol II at enhancers and promoters raises the possibility of molecular crowding given the large sizes of the complexes comprising the PIC, such as Mediator [47]. As a result, enhancers might be unable to get too close to their target promoters. Recent efforts to measure the distances between active enhancers and their target promoters suggest surprisingly large distances, on the order of a few hundred nanometer (nm). The distance between the Sonic hedgehog (*Shh*) promoter and the limb-specific ZRS enhancer in mouse limb buds, and the distance between the Sox2 promoter and the SCR enhancer in mouse embryonic stem cells both range between 200–400 nm [48,49•]. In *Drosophila* embryos, the enhancer-promoter distance during active transcription was measured to be 200–300 nm, for both an enhancer-promoter pair located across homologous alleles (transvection) and for genes located ~140 kb away from each other in *cis* [7,50]

In one particularly vivid example, the distance between an enhancer and its target promoter was actually found to increase upon transcription activation (Figure 1b). The distance between the *Shh* promoter and the neural SBE6 enhancer (~100 kb away) increased in active neural progenitor cells as compared with embryonic stem cells where the gene is silent [8••]. This result indicates that proximity is essential for a distal enhancer to activate its target gene. However, proximity does not mean direct contact, and it appears that large distances separate active enhancers and promoters during gene activity. Another study also reported an increase in enhancer mobility upon transcriptional activation, consistent with dynamic interactions between the enhancer and Pol II clusters or condensates [51]. In principle, these observations could all be explained by the hub hypothesis (Figure 1a).

Conclusions/future prospects

These are exciting times to study transcription. For many years, the field was dominated by biochemical and structural studies of the core transcription machinery. Stoichiometric complexes comprising the pre-initiation complex have been defined and visualized [52]. However, an unexpected finding is that these stoichiometric complexes form higher order associations to produce dynamic clusters or condensates. We have discussed the implications of these condensates with respect to classical models of enhancer-promoter interactions. Moving forward, there are many important questions and challenges. How do distal enhancers come into proximity with their target promoters? We have argued that molecular crowding precludes close contact, but proximity is surely important for activation. There is evidence that CTCF elements can help foster specific enhancer-promoter interactions, but it is highly likely that there are additional mechanisms [53]. Do proteins such as CTCF help nucleate the formation of activation condensates once enhancers and promoters come into proximity? And finally, are there higher-order transcription hubs containing genes scattered across large distances, similar to earlier models of transcription factories [54]? These and other outstanding issues highlight the importance of understanding transcription as a dynamic cellular process.

Conflict of interest statement

no conflict of interest.

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