

# Enhancer-promoter communication in *Drosophila* developmental gene transcription

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**ABSTRACT** Enhancers play an essential role in gene regulation by receiving cues from transcription factors and relaying these signals to modulate transcription from target promoters. Enhancer-promoter communications occur across large linear distances of the genome and with high specificity. The molecular mechanisms that underlie enhancer-mediated control of transcription remain unresolved. In this review, we focus on research in *Drosophila* uncovering the molecular mechanisms governing enhancer-promoter communication and discuss the current understanding of developmental gene regulation. The functions of protein acetylation, pausing of RNA polymerase II, transcriptional bursting, and the formation of nuclear hubs in the induction of tissue-specific programs of transcription during zygotic genome activation are considered.

**KEYWORDS:** enhancer, transcription, gene regulation, Pol II pausing, transcriptional bursting

## Introduction

The ability to express different complements of genes underlies the capacity of cells carrying the same DNA genome to form diverse cell types during multicellular development. Transcription fundamentally depends on recruitment of the RNA polymerase II (Pol II) transcriptional machinery at promoter sequences, but regulation by non-coding enhancer sequences shape the magnitude and spatio-temporal dynamics of transcriptional activity (Shlyueva *et al.*, 2014; Spitz and Furlong, 2012). Enhancers are essential *cis*-regulatory elements (CREs) that integrate regulatory signals from *trans*-acting transcription factors (TFs) into a transcriptional circuitry to drive cell type-specific programs of transcription from target promoters (Fig. 1). Enhancers are often located at large genomic distances from target promoters and must therefore communicate regulatory signals across the chromatin landscape that houses the DNA genome (Furlong and Levine, 2018). The genome is organized across multiple levels with chromosomes occupying territories within the nucleus, the segregation of chromatin into active and inactive compartments, topologically associating domains (TADs) with abundant internal interactions, including between enhancers and promoters (Jerkovic and Cavalli, 2021). Cofactors with enzymatic chromatin-modifying activities are often recruited by TFs to enhancers (Reiter *et al.*, 2017) and it is increasingly recognized that modulation of chromatin, through histone posttranslational

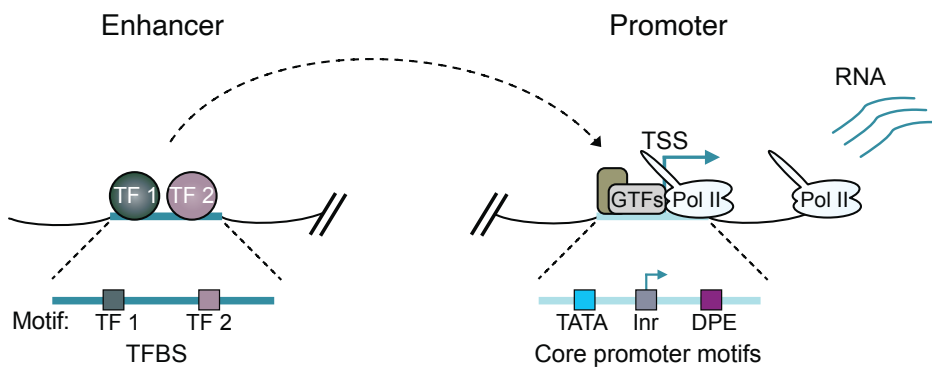
modifications (PTMs), the incorporation of histone variants, and DNA methylation, interplay with transcriptional states to ensure developmental gene regulation (Bannister and Kouzarides, 2011; Li *et al.*, 2007). While enhancers are well defined as recruitment platforms for cell type-specific TFs with dense enrichment of TF binding sites (TFBS) (Shlyueva *et al.*, 2014; Spitz and Furlong, 2012), mechanistic understanding of how enhancers communicate regulatory cues to promoters and modulate transcriptional activity is lacking (Catarino and Stark, 2018; Furlong and Levine, 2018; Karr *et al.*, 2022; Panigrahi and O'Malley, 2021).

*Drosophila melanogaster* (*Drosophila*) is a key model system for studying the molecular mechanisms underlying developmental gene regulation. During the first few hours of *Drosophila* embryogenesis, development progresses rapidly from a transcriptionally inert fertilized egg, through a series of 13 synchronous syncytial nuclear cycles (nc), to an embryo composed of ~6000 cells that undergoes zygotic genome activation (ZGA) and initiates cell type-

**Abbreviations used in this paper:** AIL, Autoinhibitory loop; AP, Anterior-posterior; CREs, Cis-regulatory elements; DI, Dorsal; DV, Dorsoventral; GRNs, Gene regulatory networks; GTFs, General transcription factors; H3K27ac, Histone H3 lysine 27 acetylation; H3K27me3, Histone H3 lysine 27 tri-methylation; nc, nuclear cycle; Pol II, RNA polymerase II; PTMs, Posttranslational modifications; TADs, Topologically associating domains; TF, Transcription factor; TFBS, TF binding sites; TSS, Transcription start site; ZGA, Zygotic genome activation.

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**Fig. 1. Enhancers and promoters integrate regulatory signals from transcription factors (TFs) to the genome to drive transcription from promoters.** The schematic depicts the core circuitry of enhancers and promoters, highlighting transcription factor binding sites (TFBS) that recruit TFs to enhancers. Additionally, core promoter motifs, such as the TATA box, initiator element (Inr), and downstream promoter element (DPE), are shown. These motifs play a crucial role in recruiting general transcription factors (GTFs) and the RNA Polymerase II (Pol II) machinery to promoters, to initiate the transcription process.

specific transcriptional programs (Harrison and Eisen, 2015; Schulz and Harrison, 2019). ZGA is orchestrated by maternally supplied mRNAs and proteins which establish an elaborate gene regulatory landscape. Chromatin becomes accessible at specific CREs and differentially bound across cells by complements of TFs, cofactors and the transcriptional machinery, while chromatin states, with specific signatures of PTMs, form (Blythe and Wieschaus, 2016; Bozek *et al.*, 2019; Calderon *et al.*, 2022; Cusanovich *et al.*, 2018; Koenecke *et al.*, 2016; Li *et al.*, 2014; Liang *et al.*, 2008; Reddington *et al.*, 2020). ZGA occurs concomitantly with formation of a complex genome organization of TADs that may facilitate close proximity between enhancers and promoters. However, the role of TADs in gene expression and the mechanisms of enhancer-promoter interactions are unclear (Furlong and Levine, 2018; Ghavi-Helm *et al.*, 2014; Hug *et al.*, 2017; Ogiyama *et al.*, 2018; Schoenfelder and Fraser, 2019).

Gene regulatory networks (GRNs) specify cell identities along the dorsoventral (DV) and anterior-posterior (AP) body axes to spatially coordinate formation of the germ layers and organize the future body plan (Ma *et al.*, 2016; Stein and Stevens, 2014). These well-characterized *Drosophila* GRNs are valuable for investigating how gene regulatory mechanisms converge to produce cell-type specific transcriptional programs. The importance of better understanding the molecular mechanisms of enhancer function is underscored by the presence of disease-associated variants within non-coding sequences (Rickels and Shilatifard, 2018; Zaugg *et al.*, 2022), the pervasiveness of mutations to chromatin-modifying proteins in cancer (Flavahan *et al.*, 2017) and the relevance of transcriptional and chromatin alterations in aging (López-Otín *et al.*, 2013). In this review, we discuss current understanding of developmental gene regulation and particularly draw on research in *Drosophila* focused on uncovering the molecular mechanisms governing enhancer-promoter communication and enhancer-mediated control of transcription.

### Enhancers operate in complex regulatory landscapes

Enhancer activity was initially demonstrated for a sequence element from the SV40 virus genome, capable of increasing the magnitude of transcription from the rabbit  $\beta$ -globin gene in HeLa cells when positioned more than 1 kb upstream or downstream from the transcription start site (TSS) (Banerji *et al.*, 1981). Soon after, enhancer activity was detected for endogenous sequences from the eukaryotic genome (Banerji *et al.*, 1983; Gillies *et al.*, 1983). Enhancers have evolved to play a key role in orchestrating

developmental transcriptional programs (Long *et al.*, 2016; Shlyueva *et al.*, 2014; Spitz and Furlong, 2012), vastly outnumbering protein-coding genes within eukaryotic genomes. The enhancer regulatory code is highly conserved across metazoans, with enhancer activities of many non-coding sequences preserved across distant species (Pennacchio *et al.*, 2006; Wong *et al.*, 2020). The functional hallmarks of enhancers include the capacity to modulate transcription in an orientation independent manner, operate at large genomic distances from the target promoter, and the ability to recapitulate their activity independent of the sequence context (reviewed in Shlyueva *et al.*, 2014). *In vivo* reporter assay validation of sequences predicted to have enhancer activity remains essential for establishing functionality, and many candidate enhancers have not yet been validated (Kvon *et al.*, 2014; Pennacchio *et al.*, 2006; Shlyueva *et al.*, 2014; Smith *et al.*, 2023).

### Chromatin restricts enhancer accessibility

The communication of regulatory signals occurs across chromatin, and genomic approaches to predict sequences with enhancer activity based on DNA and chromatin features have been useful for identifying enhancers genome-wide and inferring cell type-specific enhancer activities (Encode Project Consortium, 2012; Negre *et al.*, 2011; The modEncode Consortium *et al.*, 2010). Active enhancers are characterized by accessible chromatin depleted of nucleosomes to allow for DNA binding by TFs. Genome-wide profiling has revealed that chromatin accessibility is dynamically regulated across cell types and correlates with TF binding (Fig. 2A) (Boyle *et al.*, 2008; Li *et al.*, 2011; Pique-Regi *et al.*, 2011; Shlyueva *et al.*, 2014; Spitz and Furlong, 2012; Thurman *et al.*, 2012). Elevated chromatin accessibility is also a feature of other CREs. At active promoters, nucleosome depletion is important for assembly of the transcriptional machinery (Cairns, 2009) and Pol II may compete with nucleosomes to maintain an accessible state (Fig. 2A) (Core and Adelman, 2019; Gilchrist *et al.*, 2010; Levine, 2011). Polycomb response elements (PREs), the recruitment sites of Polycomb group (PcG) proteins that are responsible for transcriptional silencing of developmental genes across eukaryotes (Schuettengruber *et al.*, 2017), also exhibit low nucleosome density (Hunt *et al.*, 2022; Mito *et al.*, 2007; Schuettengruber *et al.*, 2009; Schwartz *et al.*, 2006).

A major function of housing the genome in chromatin is to prevent spurious transcription (Kornberg and Lorch, 2020). Therefore, factors capable of selectively producing nucleosome-depleted regions play a key role in gene regulation. A subset of TFs, known as pioneer factors, have the capacity to initiate the opening of

compact chromatin (Balsalobre and Drouin, 2022; Spitz and Furlong, 2012). Pioneer factors can interact with nucleosomal DNA and recruit chromatin remodelers to deplete nucleosomes at these sites, providing DNA access for non-pioneer TFs. In this manner, the maternally supplied pioneer factor Zelda (Zld) selectively establishes chromatin accessibility at important CREs for ZGA in *Drosophila* (Harrison *et al.*, 2011; Liang *et al.*, 2008; Nien *et al.*, 2011; Schulz and Harrison, 2019; Sun *et al.*, 2015). Zld orchestration of ZGA is supported by several other TFs with pioneer-like activities, including the mitotic bookmark GAGA factor (GAF) (Bellec *et al.*, 2022; Gaskill *et al.*, 2021), Odd-paired (Opa) (Koromila *et al.*, 2020; Soluri *et al.*, 2020) and CLAMP (Duan *et al.*, 2021).

**Active enhancers are marked by H3K27 acetylation**

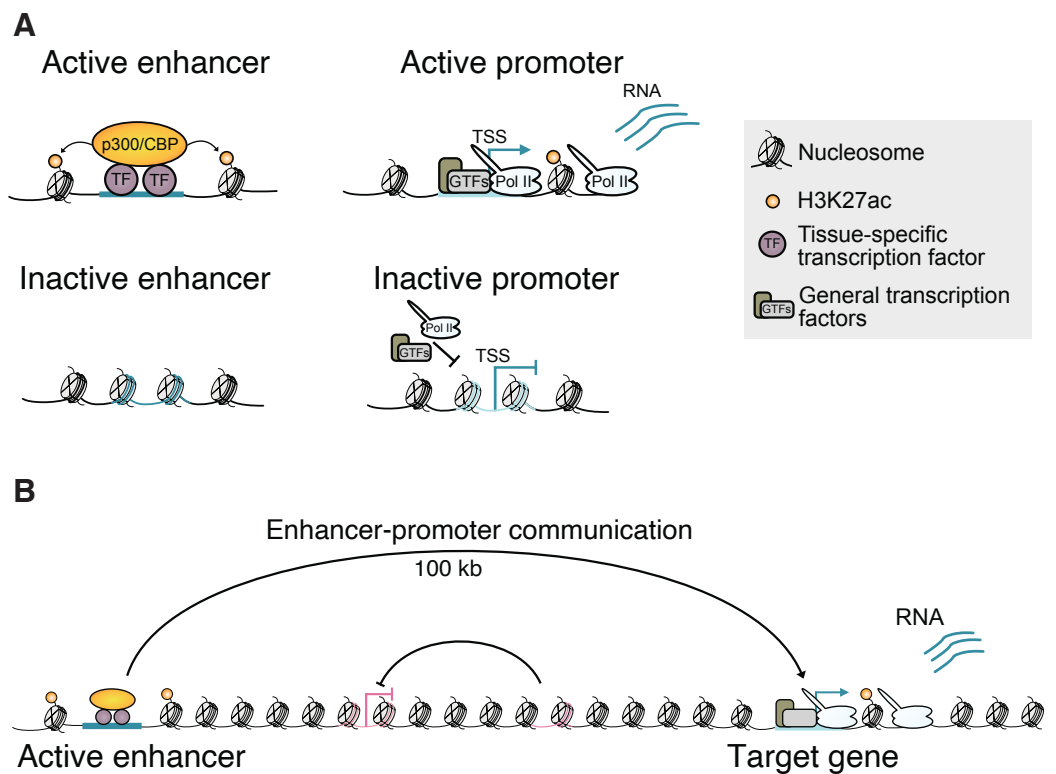
Specific signatures of histone PTMs are important markers for identifying enhancers and predicting enhancer activity states genome-wide (Shlyueva *et al.*, 2014). Acetylation of histone H3 lysine 27 (H3K27ac) is detected at nucleosomes flanking enhancers, and its enrichment correlates with transcriptional activity from associated genes (Fig. 2A), allowing the mark to distinguish active from inactive or poised enhancers (Bonn *et al.*, 2012; Creyghton *et al.*, 2010; Heintzman *et al.*, 2009; Rada-Iglesias *et al.*, 2011). Occupancy of p300/CBP, the histone acetyltransferases responsible for depositing H3K27ac (Tie *et al.*, 2009), has also been used to predict enhancers (Heintzman *et al.*, 2007; Visel *et al.*, 2009; Xi *et al.*, 2007). Nevertheless, p300/CBP recruitment and acetyltransferase activity can be uncoupled because strong occupancy also occurs at hypoacetylated regions enriched for the repressive PcG mark trimethylated H3K27 (H3K27me3) (Holmqvist *et al.*, 2012; Hunt *et al.*, 2022; Philip *et al.*, 2015; Rada-Iglesias *et al.*, 2011). H3K27ac marks both active enhancers and promoters, but mono- and tri-methylation of H3K4 distinguish enhancers and promoters, respectively (Heintzman *et al.*, 2007; Rada-Iglesias *et al.*, 2011; Shlyueva *et al.*, 2014). Whether histone acetylation plays a direct causative role in gene activation remains largely unclear (Henikoff and Shilatifard, 2011; Millán-Zambrano *et al.*, 2022). Histone acetylation may indirectly influence transcription by supporting the recruitment of effector proteins that recognize acetylated lysines, such as the coactivator bromodomain-containing protein 4 (BRD4) (Dey *et al.*, 2003; Fujisawa and Filippakopoulos, 2017; Millán-Zambrano *et al.*, 2022).

Distinct motif compositions and chromatin features shape the specificities of enhancers and promoters, influencing cofactor

compatibilities (Haberle *et al.*, 2019; Neumayr *et al.*, 2022). Different chromatin remodelers are necessary for developmental and housekeeping transcriptional programs in *Drosophila* cells (Hendy *et al.*, 2022). Remarkably, enhancers can operate long-range, spanning hundreds of kilobases (kb) from their target promoter, often bypassing intervening promoters (Fig. 2B). This implies a topological change in 3D organization, involving chromatin fiber folding, to confer specificity and allow proximity or physical contact for enhancer-promoter communication. However, while enhancers are well-defined compositionally by the TFBS they encode and specific chromatin features (Shlyueva *et al.*, 2014; Spitz and Furlong, 2012), how signals are communicated from enhancers to target promoters, modulating transcriptional activity, remains unclear (Catarino and Stark, 2018; Furlong and Levine, 2018; Karr *et al.*, 2022; Panigrahi and O'Malley, 2021).

**Enhancer-mediated control of *Drosophila* developmental transcription**

Studies of *Drosophila* embryonic patterning have unveiled how enhancers coordinate developmental gene expression (Irizarry and Stathopoulos, 2021; Small and Arnosti, 2020). The precise stripes of pair-rule gene expression across the AP axis of the early embryo are driven by multiple enhancers, each encoding different combinations of TFBS (Levine, 2010; Small and Arnosti, 2020; Small *et al.*, 1992; Stanojevic *et al.*, 1991). These enhancers act additively to form the overall expression pattern. The involvement of multiple enhancers with partially redundant activity, known as



**Fig. 2. Enhancers operate across chromatin to influence transcription from target promoters.** (A) Schematic showing active and inactive enhancer and promoter chromatin states. (B) Communication between enhancers and promoters often occurs over large genomic distances with specificity, enabling these long-range interactions to bypass intervening enhancers and promoters.

shadow enhancers, in directing gene expression patterns may contribute to phenotypic robustness (Cannavò *et al.*, 2016; Frankel *et al.*, 2010; Hong *et al.*, 2008a; Osterwalder *et al.*, 2018; Perry *et al.*, 2010). *Drosophila* embryogenesis has been a key model for systematically characterizing non-coding sequences with developmental enhancer activity *in vivo* (Kvon *et al.*, 2014). Despite its gene-dense nature relative to mammalian genomes, long-range interactions are widespread in the *Drosophila* genome. Many enhancer-promoter interactions occur at distances of more than 10 kb, with the majority spanning at least several kb. This separation still requires a topological mechanism to provide the necessary physical proximity for enhancer-bound TFs and cofactors to influence the transcriptional machinery at the promoter (Furlong and Levine, 2018; Ghavi-Helm *et al.*, 2014; Hou *et al.*, 2012; Hug *et al.*, 2017; Sexton *et al.*, 2012). The extensive validation of enhancers and widespread embryonic formation of enhancer-promoter interactions make *Drosophila* embryogenesis well suited for dissecting the mechanisms of enhancer-mediated gene activation.

### ***Drosophila* dorsal-ventral patterning**

Position-dependent transcriptional programs define cell identities along the *Drosophila* embryonic DV axis in response to a nuclear gradient of the maternally-supplied Rel family TF Dorsal, peaking ventrally and progressively decaying dorsally (Fig. 3A) (Hong *et al.*, 2008b; Irizarry and Stathopoulos, 2021; Stathopoulos and Levine, 2002; Stein and Stevens, 2014). A cascade of maternal effect genes relays DV polarity to the syncytial blastoderm through ventrally restricted activation of Toll signaling, releasing Dorsal from an inactive cytoplasmic complex to enter nuclei (Belvin *et al.*, 1995; Roth *et al.*, 1989; Rushlow *et al.*, 1989; Steward, 1987, 1989). High nuclear Dorsal ventrally activates mesoderm-specific genes like *twist* (*twi*) and *snail* (*sna*) to form mesoderm, while intermediate and low levels of nuclear Dorsal laterally induce neuroectoderm genes, including *intermediate neuroblasts defective* (*ind*) and *brinker* (*brk*), directing neuroectoderm formation. *Sna*, with the CtBP and Ebi corepressors, represses neuroectoderm-specific genes in the mesoderm (Fig. 3A) (Nibu *et al.*, 1998; Qi *et al.*, 2008). Dorsally located cells lacking nuclear Dorsal activate genes like *decapentaplegic* (*dpp*) and *tolloid* (*tld*) for dorsal ectoderm patterning. Besides acting as a transcriptional activator for mesoderm- and neuroectoderm-specific genes, Dorsal, along with the transcriptional repressor Capicua and corepressor Groucho, confines the expression of dorsal ectoderm-specific genes to dorsally located cells (Dubnicoff *et al.*, 1997; Papagianni *et al.*, 2018). Dpp/BMP signaling further defines cell types along the dorsal ectoderm (Ashe *et al.*, 2000; Ferguson and Anderson, 1992; Hamaratoglu *et al.*, 2014). The Dorsal gradient forms during the first 90 minutes of embryogenesis and is active between nc 10-14 (Lieberman *et al.*, 2009; Reeves *et al.*, 2012). It is during this critical developmental window that the TF cue is received by enhancers of the DV GRN and communicated to modulate transcription from the promoters of DV-regulated genes.

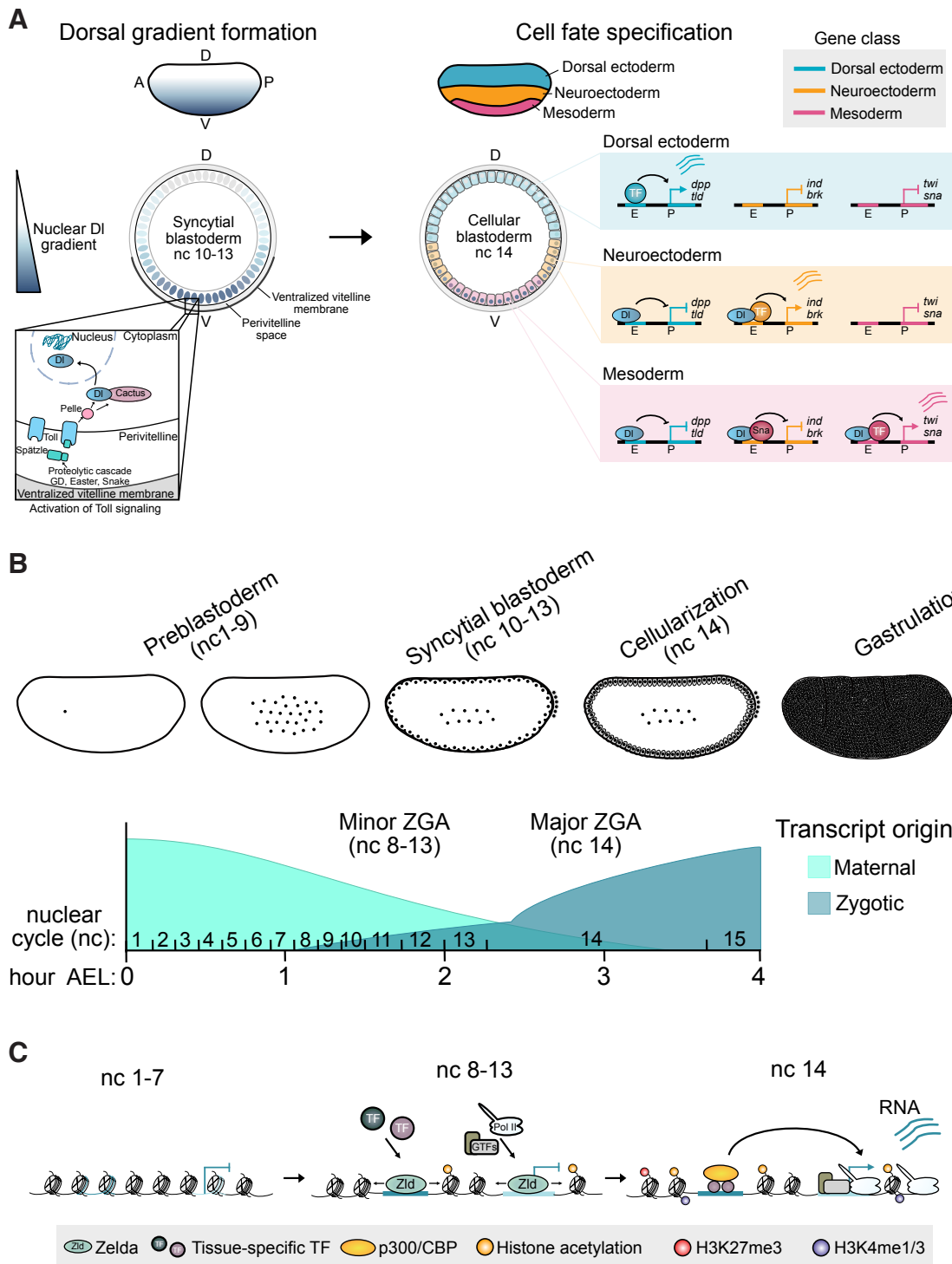
More than 100 DV-regulated genes, many encoding developmental regulators, and 200-400 enhancers have been identified. Initial interrogation of the DV GRN involved genetic analyses (reviewed in Stein and Stevens, 2014), followed by genomic approaches including bioinformatics mapping of Dorsal binding

sites and whole-genome microarray analysis of the transcriptome and Dorsal occupancy in mutant embryos with uniform DV cell fates (Biemar *et al.*, 2006; Markstein *et al.*, 2002; Stathopoulos *et al.*, 2002; Zeitlinger *et al.*, 2007b). Recently, next generation sequencing methods have captured spatially- and temporally-resolved transcriptional and epigenomic landscapes during *Drosophila* embryogenesis (Blythe and Wieschaus, 2016; Bozek *et al.*, 2019; Chen *et al.*, 2013; Holmqvist *et al.*, 2012; Hunt *et al.*, 2024; Ing-Simmons *et al.*, 2021; Koenecke *et al.*, 2016; Koenecke *et al.*, 2017; Li *et al.*, 2014; Lott *et al.*, 2011), including at the single-cell scale (Calderon *et al.*, 2022; Cusanovich *et al.*, 2018; Hunt *et al.*, 2024; Ing-Simmons *et al.*, 2021; Karaiskos *et al.*, 2017; Reddington *et al.*, 2020).

The threshold-dependent model proposes that spatially-regulated expression along the DV axis is achieved by integration of the Dorsal TF signal at enhancers responding to different Dorsal levels, determined by the affinity and organization of the binding sites they encode (Reeves and Stathopoulos, 2009; Rusch and Levine, 1996; Stathopoulos and Levine, 2002, 2004). While Dorsal initiates DV patterning, and enhancer binding site affinity correlates with positional expression (Papatsenko and Levine, 2005), precise expression domains of DV-regulated genes involve Dorsal acting in concert with other tissue-specific TFs, repressors and cofactors (Holmqvist *et al.*, 2012; Kosman *et al.*, 1991; Mannervik *et al.*, 1999; Zeitlinger *et al.*, 2007b). DV patterning constitutes one of the best-characterized GRNs in nature and has been a major model system for illuminating enhancer-mediated control of developmental transcription.

### **Interplay between enhancer chromatin states and tissue-specific transcription in *Drosophila***

During early *Drosophila* embryogenesis, chromatin rapidly changes in the lead-up to ZGA, transitioning from a presumed highly condensed and hypoacetylated state to a heterogeneous landscape of distinct chromatin states with selective accessibility and the deposition of histone PTMs at specific genomic regions (Fig. 3 B,C) (Blythe and Wieschaus, 2016; Bozek *et al.*, 2019; Harrison *et al.*, 2011; Hunt *et al.*, 2024; Li *et al.*, 2014; Schulz and Harrison, 2019). Histone acetylation by p300/CBP accumulates at important enhancers and promoters associated with Zld activity (Harrison *et al.*, 2011; Hunt *et al.*, 2024; Li *et al.*, 2014). H3K27ac is enriched at DV enhancers and promoters, and differential H3K27ac enrichment between DV cell types has been used to predict DV-regulated enhancers (Boija and Mannervik, 2016; Ing-Simmons *et al.*, 2021; Koenecke *et al.*, 2016). Integrating multiple indicators of the epigenome (H3K27ac and p300/CBP enrichment and chromatin accessibility) predicted DV-regulated enhancers more accurately than each could individually (Hunt *et al.*, 2024). Interestingly, the strength of this tissue-specific enhancer chromatin state, consisting of elevated H3K27ac, p300/CBP, and chromatin accessibility, was highly predictive of the magnitude of tissue-specific transcription from associated DV-regulated genes. The DV promoter chromatin state, as defined by these epigenomic markers, was less tissue-specific and therefore not as effective at predicting differential transcription as the enhancer. Nevertheless, while these studies have uncovered chromatin features that strongly correlate with transcriptional states, much remains ambiguous regarding functional involvement.



**Fig. 3. Dorsal (DV) patterning of *Drosophila* embryogenesis and chromatin landscape establishment during zygotic genome activation (ZGA).** (A) The establishment of the Dorsal (Dl) TF nuclear gradient occurs in the syncytial blastoderm during nuclear cycle (nc) 10-13. This is facilitated by ventrally restricted activation of the Toll signaling pathway, leading to position-dependent cell fate specification of mesoderm, neuroectoderm, and dorsal ectoderm across the DV axis at nc 14. Cell identity is determined by tissue-specific transcription programs induced through enhancers responsive to different concentrations of Dl. Dorsal ectoderm-specific genes like *dpp* and *tld* are restricted to the most dorsally located cells due to Dorsal-mediated repression in lateral and ventral cells (Dubnicoff *et al.*, 1997; Papagianni *et al.*, 2018). Mesoderm-specific genes, including *sna* and *twi*, are expressed in ventrally-located cells with high levels of nuclear Dl, while neuroectoderm-specific genes like *ind* and *brk* are activated by Dl in lateral regions

but not ventrally due to repression by *Sna* with the CtBP and Ebi corepressors (Nibu *et al.*, 1998; Qi *et al.*, 2008). Robust induction of the transcriptional programs patterning the DV axis depends on Dl acting in concert with other tissue-specific TFs, repressors, and cofactors. (B) A schematic of the stages of early *Drosophila* embryogenesis, illustrating nuclei (represented by black dots) dividing through rapid nuclear cycles. Nuclear divisions occur first within the center of the embryo in the preblastoderm stages (nc 1-9) before nuclei migrate to the periphery during the syncytial blastoderm stages (nc 10-13). At the periphery, nuclei cellularize to form the cellular blastoderm (nc 14) before undergoing gastrulation. The exchange of transcriptional control from maternal to zygotic during the minor and major waves of ZGA is also depicted. The hours after egg laying (AEL) are indicated across the trajectory and the corresponding nuclear cycles shown. (C) Changes to the chromatin landscape at an enhancer and promoter that become transcriptionally active at nc 14. In the lead-up to transcriptional induction, regulatory sequences are made accessible by the pioneer factor Zelda, facilitating the recruitment of TFs and the transcriptional machinery. Chromatin states that are linked to transcription, such as the H3K27ac histone modification, also begin to accumulate.

## Functions of histone acetylation in transcription

Whether histone acetylation plays an instructive role in gene activation is unclear (Henikoff and Shilatifard, 2011; Millán-Zambrano *et al.*, 2022). Early *in vitro* studies noted that histone acetylation suppressed the intrinsic inhibitory effect of histones on transcription from chromatinized DNA templates (Allfrey *et al.*, 1964). This effect is understood to be mediated by acetylation-induced alterations to nucleosomal electrostatic interactions, leading to decompaction and increased amenability for transcription (Fenley *et al.*, 2010). However, histone tail PTMs are considered unlikely to impact nucleosome dynamics and may instead influence transcription indirectly by providing a binding platform for effector proteins (Millán-Zambrano *et al.*, 2022). Consistently, the coactivator BRD4 is recruited to *Drosophila* DV enhancers and promoters in a tissue-specific manner correlating with H3K27ac and transcriptional activation (Hunt *et al.*, 2024). H3K27ac was also recently observed to enhance cooperative binding at human OCT4-pioneered sites (Sinha *et al.*, 2023). Zelda binding at H3K27ac-marked DV and non-DV enhancers before ZGA in *Drosophila* (Hunt *et al.*, 2024; Li *et al.*, 2014) raises speculation about a similar association in the early embryo.

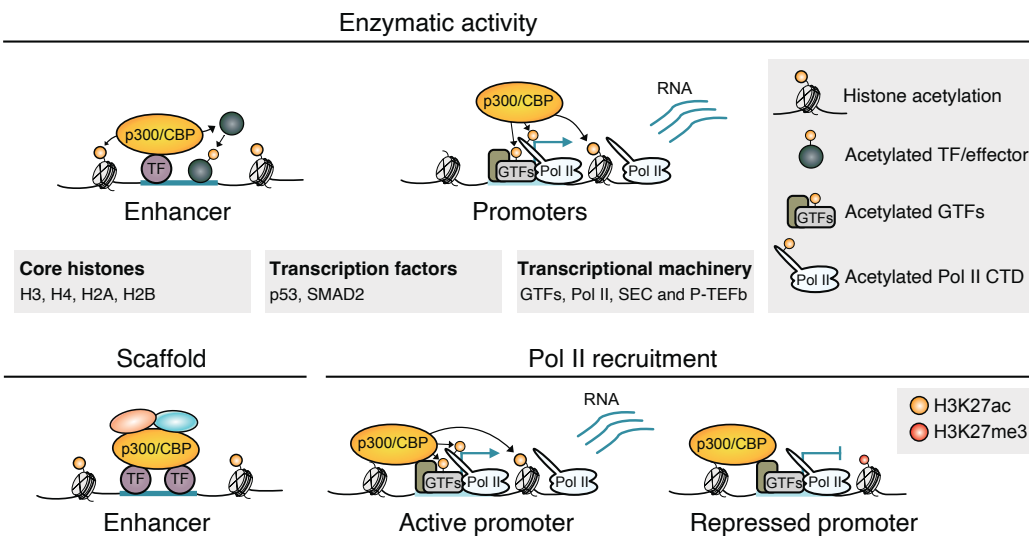
## Acetylation of non-histone proteins

p300/CBP, like many other chromatin-modifiers, is a multifunctional protein with enzymatic and non-enzymatic activities, complicating the attribution of observed effects to specific functions (Fig. 4) (Bedford and Brindle, 2012; Dorigi *et al.*, 2017; Hunt *et al.*, 2022; Morgan and Shilatifard, 2020; Rickels *et al.*, 2017). In addition to acetylating histones, the acetyltransferase activity of p300/CBP targets non-histone proteins, such as TFs, coactivators, and effectors of important signaling pathways (Weinert *et al.*, 2018).

Acetylation can influence protein function in diverse ways, including affecting protein stability, localization, protein-protein interactions, and DNA-binding ability (Spange *et al.*, 2009). p300/CBP is equipped with a bromodomain and various other protein-protein interaction domains that may mediate non-enzymatic functions (Dancy and Cole, 2015). These functions could include acting as a scaffold to facilitate the assembly of protein complexes (Fig. 4) (Chan and La Thangue, 2001).

p300/CBP is also found at promoters, where it interacts with the Pol II transcriptional machinery (Boija *et al.*, 2017; Cho *et al.*, 1998; Heintzman *et al.*, 2007). At promoters, p300/CBP may influence transcription through enzymatic and non-enzymatic functions (Fig. 4) (Boija *et al.*, 2017; Narita *et al.*, 2021), aiding in the formation of the pre-initiation complex (PIC) via interactions with TFIIB (Kwok *et al.*, 1994) and modulating Pol II promoter release, potentially by acetylating the transcriptional machinery and the +1 nucleosome (Boija *et al.*, 2017; Narita *et al.*, 2021; Schröder *et al.*, 2013; Stasevich *et al.*, 2014). The presence of p300/CBP and the Pol II transcriptional machinery at promoters of genes devoid of acetylation and productive transcription suggests its different functions can be partitioned (Hunt *et al.*, 2022).

The accumulation of p300/CBP histone acetylation around ZGA is conserved in flies, zebrafish and mice (Bogdanović *et al.*, 2012; Dahl *et al.*, 2016; Li *et al.*, 2014; Schulz and Harrison, 2019). Modulation of p300 acetyltransferase activity has been reported to disrupt ZGA in zebrafish (Chan *et al.*, 2019; Miao *et al.*, 2022; Sato *et al.*, 2019). Interestingly, although p300/CBP is essential for ZGA in *Drosophila*, ZGA can occur in embryos with catalytically inactive p300/CBP (Ciabrelli *et al.*, 2023). Moreover, p300/CBP protein depletion, but not acetyltransferase activity inhibition, compromises chromatin accessibility (Hogg *et al.*, 2021; Hunt *et al.*, 2022; Vannam *et al.*, 2021), further highlighting the crucial non-enzymatic roles of p300/CBP activities in gene regulation.



**Fig. 4. Functions of p300/CBP in gene regulation.** The chromatin-modifier p300/CBP regulates transcription through enzymatic acetyltransferase activity, but also has non-enzymatic activities. p300/CBP acetylates lysine residues on all four core histones, including H3K27ac to mark active enhancers and promoters (Feller *et al.*, 2015; Weinert *et al.*, 2018), but also acetylates diverse non-histone proteins, including TFs, transcriptional regulators, and effectors of signaling pathways (Weinert *et al.*, 2018). p300/CBP also enzymatically targets important components of the transcriptional machinery at promoters, including GTFs, Pol II, the super elongation complex (SEC) and P-TEFb, which alongside acetylation of the +1 nucleosome, may stimulate Pol II release from the promoter into productive transcription

(Boija *et al.*, 2017; Narita *et al.*, 2021; Schröder *et al.*, 2013; Stasevich *et al.*, 2014). Non-enzymatic activities of p300/CBP in gene regulation could include scaffolding to help bring together TFs and other transcriptional regulators into large assemblies at target loci (Chan and La Thangue, 2001) and supporting assembly of the transcriptional machinery at promoters by interacting with TFIIB (Kwok *et al.*, 1994). p300/CBP support of Pol II recruitment also occurs at the promoters of certain genes in repressive, H3K27me3-enriched chromatin, where productive transcription is lacking (Hunt *et al.*, 2022).

Targeting H3K27 directly by replacing it with non-modifiable residues (H3K27A/R) does not compromise gene activation in *Drosophila* and mouse embryonic stem cells (mESCs), indicating that p300/CBP functions aside from acetylating H3K27 are important (Leatham-Jensen *et al.*, 2019; McKay *et al.*, 2015; Pengelly *et al.*, 2013; Sankar *et al.*, 2022; Zhang *et al.*, 2020). p300/CBP is proposed to act as an "acetyl spray" that targets lysines across the tails of histones (Feller *et al.*, 2015; Weinert *et al.*, 2018). Supporting this spray-like activity, histone H2B tail acetylation by p300/CBP can also predict active enhancers (Narita *et al.*, 2023). It is possible that acetylation of neighboring histone tail residues confers functional redundancy or is a by-product of enzymatic activity directed at non-histone proteins, which also affects residues on local histones. The acetyltransferase activity of p300/CBP is tightly regulated by a lysine-rich autoinhibitory loop (AIL) that, in a deacetylated state, prevents substrate access to the active site (Thompson *et al.*, 2004). Dimerization of TFs and binding of enhancer RNAs (eRNAs) are proposed to trigger displacement of the p300/CBP AIL, allowing for enzymatic activity to occur (Bose *et al.*, 2017; Ortega *et al.*, 2018). The *Drosophila* PcG protein Pc and its mammalian CBX orthologs directly interact with the p300/CBP AIL to block acetyltransferase activity, potentially preventing turnover from transcriptionally repressive to active states (Tie *et al.*, 2016). This aligns with the detection of p300/CBP at hypoacetylated sites (Holmqvist *et al.*, 2012; Hunt *et al.*, 2022; Philip *et al.*, 2015; Radalglesias *et al.*, 2011) and supports localized multi-step enzymatic activation of p300/CBP that is uncoupled from chromatin recruitment. The transcription of DV-regulated genes anti-correlates with H3K27me<sub>3</sub>, suggesting that local enzymatic inhibition of CBP by PcGs may help ensure tissue-specific transcription (Hunt *et al.*, 2024; Koenecke *et al.*, 2017). CBP binding across cell types of the DV axis indicates the potential for distinct recruitment and enzymatic activity state dynamics at enhancers and promoters, where enhancer occupancy is highly tissue-specific and correlates with tissue-specific TF presence, H3K27ac, and transcription (Hunt *et al.*, 2024). In contrast, CBP promoter binding is less tissue-specific, with residual levels persisting at promoters in inactive states.

An intriguing mechanism for enhancer-promoter communication, termed the TF activity gradient (TAG) model, proposes that acetylated TFs produced by enhancer-localized enzymatic p300/CBP activity diffuse to target promoters in close proximity, where they increase the transcriptional output (Karr *et al.*, 2022). In this model, histone deacetylases (HDAC), chromatin modifiers that also target non-histone proteins (Glozak *et al.*, 2005), deacetylate TFs to spatially limit the diffusing acetylated TF signal. Indeed, HDACs are recruited to active promoters in metazoans, where they may attenuate acetylated TF signals (Filion *et al.*, 2010; Rincon-Arango *et al.*, 2012; Wang *et al.*, 2008). Concentrations of p300/CBP and other coactivators appear to be limiting in cells, with the number of molecules being far outnumbered by HDACs (Gillespie *et al.*, 2020). In *Drosophila* cell culture, pharmacological HDAC inhibition rapidly elevates histone acetylation levels at active promoters and correlates with Pol II promoter release (Vaid *et al.*, 2020). These findings support HDAC-mediated attenuation of acetylated TF signals as they diffuse from enhancers and the maintenance of the majority of nuclear p300/CBP in an inactive state (Karr *et al.*, 2022).

Considering the uncertainty surrounding the direct role of histone acetylation in transcription, there is significant focus on identifying factors that undergo function-altering acetylation. This criteria may

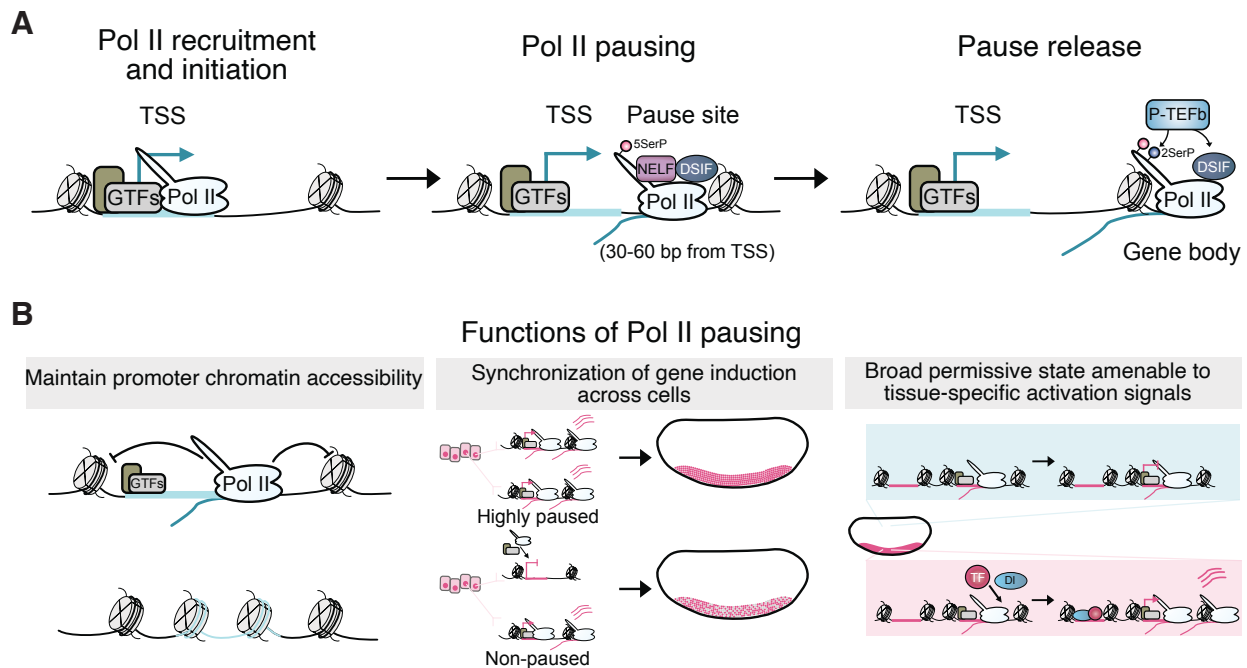
be met by the positive transcription elongation factor-b complex (P-TEFb), which stimulates Pol II elongation across gene bodies to produce transcripts (Fujinaga *et al.*, 2023; Jonkers and Lis, 2015) and is recruited to promoters by DNA-binding TFs, including NF- $\kappa$ B (Barboric *et al.*, 2001; Danko *et al.*, 2013), and BRD4 (Dey *et al.*, 2003; Jang *et al.*, 2005; Yang *et al.*, 2005). Both BRD4 and P-TEFb are recruited to *Drosophila* DV enhancers and promoters in a tissue-specific manner (Hunt *et al.*, 2024) and undergo p300/CBP-dependent acetylation in mammalian cells (Weinert *et al.*, 2018). Acetylation of P-TEFb marks its active state, free from inactive sequestration within the 7SK small nuclear ribonucleoprotein (7SK snRNP) complex (Cho *et al.*, 2009). In summary, several observations from enhancer-driven transcription during DV patterning align with a model of enhancer-promoter communication where p300/CBP actively participates. *Drosophila* embryogenesis provides a conducive *in vivo* setting for delineating the genomic distribution of both enzymatically active and inactive p300/CBP. Further investigations can focus on unravelling the regulatory mechanisms governing the loading BRD4 and P-TEFb at promoters by enhancers, and establishing whether their acetylation occurs in a functionally significant manner.

### How do enhancers influence transcriptional activity at promoters?

When enhancer-promoter communication occurs, signals of activation must be integrated into the transcriptional circuitry by modulating the activity of the Pol II transcriptional machinery. Transcription is a complex, multi-step process, and post-recruitment control of Pol II entry into elongation has emerged as a major rate-limiting checkpoint that controls the transcriptional output (Core and Adelman, 2019; Jonkers and Lis, 2015). At many metazoan genes, Pol II efficiently initiates transcription but pauses in the promoter-proximal region, typically 30-60 bp downstream of the TSS (Fig. 5A). This pausing of Pol II is highly stable, with most half-lives at the pause site ranging between 5-15 minutes, providing a window of time for regulatory signals from enhancers to exert their influence on the transcriptional cycle (Henriques *et al.*, 2013; Jonkers *et al.*, 2014; Shao and Zeitlinger, 2017). The release of Pol II from the pause site into productive elongation is tightly controlled by factors that either maintain pausing or stimulate promoter escape (Core and Adelman, 2019). P-TEFb is understood to promote pause release by phosphorylating specific residues on the Pol II C-terminal domain (CTD) and counteracting factors involved in maintaining pausing, such as Spt5, although the precise mechanism leading to elongation is not yet fully understood (Fujinaga *et al.*, 2023; Price, 2000; Yamada *et al.*, 2006).

### Promoter-proximal Pol II pausing at developmental genes

*Drosophila* early embryogenesis has played a pivotal role in elucidating the role of pausing in the coordination of tissue-specific transcription during development. Pausing was initially proposed as a mechanism to prime inducible genes for rapid signal-responsive transcriptional induction (Gilmour and Lis, 1986; Levine, 2011; Rougvie and Lis, 1988), but genome-wide mapping of Pol II occupancy subsequently uncovered the prevalence of pausing at developmental genes across metazoans (Core *et al.*, 2008; Muse *et al.*, 2007; Nechaev *et al.*, 2010; Zeitlinger *et al.*, 2007a).



**Fig. 5. Pol II pausing and its functions during *Drosophila* embryogenesis.** (A) Schematic depicting the steps in the transcriptional cycle, showing the recruitment of Pol II, promoter-proximal Pol II pausing, and release of Pol II into productive elongation. (B) Proposed functions of Pol II pausing in gene regulation during development include aiding in repelling nucleosomes from promoter DNA to maintain accessible promoter chromatin (Gilchrist *et al.*, 2010). Pol II pausing is also suggested to synchronize gene induction across cells of a tissue (Boettiger and Levine, 2009; Day *et al.*, 2016; Gaertner and Zeitlinger, 2014; Lagha *et al.*, 2013; Levine, 2011; Ramalingam *et al.*, 2021; Saunders *et al.*, 2013). Additionally, pausing may provide a broadly permissive promoter state that is receptive to tissue-specific signals triggering pause release and gene induction (Gaertner and Zeitlinger, 2014).

Consistent with serving a more fundamental and widespread role, pausing has been shown to repel nucleosomes at promoters to maintain nucleosome depletion and ensure promoter DNA is kept accessible for efficient cycles of Pol II recruitment and transcription (Fig. 5B) (Core and Adelman, 2019; Gaertner and Zeitlinger, 2014; Gilchrist *et al.*, 2010; Levine, 2011). Pausing may also help synchronize the induction of transcription across tissues and reduce transcriptional noise by attenuating variation in Pol II recruitment between cells (Boettiger and Levine, 2009; Day *et al.*, 2016; Gaertner and Zeitlinger, 2014; Lagha *et al.*, 2013; Levine, 2011; Ramalingam *et al.*, 2021; Saunders *et al.*, 2013). Highly stable paused Pol II at the pause site can inhibit new initiation events from taking place, potentially controlling the refractory period between rounds of transcription (Shao and Zeitlinger, 2017).

Pausing has been proposed to constitute a broadly permissive state across cell types that is amenable to restricted signals of gene induction (Fig. 5B) (Gaertner and Zeitlinger, 2014). While the majority of paused genes are transcriptionally active, Pol II is recruited and pauses at developmental promoters in inactive states, including DV-regulated genes during early embryogenesis (Hunt *et al.*, 2024; Zeitlinger *et al.*, 2007a). Pausing also occurs at the promoters of many H3K27me3-enriched PcG repressed developmental genes in mammalian embryonic stem cells (Bernstein *et al.*, 2006; Kanhere *et al.*, 2010; Stock *et al.*, 2007) and *Drosophila* (Enderle *et al.*, 2011; Hunt *et al.*, 2022). Therefore, pausing appears to be a major control point of the transcriptional cycle where activating and repressing signals converge. Paused Pol II can persist at genes that have been downregulated, marking their prior activation (Gaertner *et al.*, 2012). The presence of paused Pol II at DV-regulated genes in both active and inactive tissues seems to result from its recruitment before

the establishment of the Dorsal gradient in largely transcriptionally quiescent naïve cells. These promoters may be pioneered with accessibility at this early stage of development by Zelda and are highly enriched for core promoter elements capable of recruiting the transcriptional machinery (Blythe and Wieschaus, 2016; Harrison *et al.*, 2011; Hunt *et al.*, 2024). These findings support tissue-specific transcription in the early embryo involving the establishment of a paused state broadly, rendering cells receptive to spatially restricted developmental cues. In the case of DV patterning, the developmental cue is tissue-specific enhancer activation by Dorsal, which may modulate BRD4 and P-TEFb activity through p300/CBP-catalyzed acetylation to promote pause release. *Drosophila* AP patterning genes also recruit paused Pol II in naïve cells, and mammalian Pol II is pre-loaded at promoters before ZGA (Liu *et al.*, 2020), suggesting a conserved process for developmental gene induction.

Recently, the role of the Integrator complex in the regulation of Pol II pause release to control productive transcriptional elongation has been characterized in mammals (Fianu *et al.*, 2021; Stein *et al.*, 2022; Vervoort *et al.*, 2021; Wang *et al.*, 2023). Integrator drives premature termination through two enzymatic activities: an endonuclease that cleaves nascent RNA and a protein phosphatase that removes stimulatory phosphorylation associated with Pol II pause release and productive elongation (reviewed in Wagner *et al.*, 2023). Targeted and rapid protein depletion has revealed that promoter H3K4me3 recruits the integrator complex subunit 11 (INTS11) endonuclease (Wang *et al.*, 2023). Analyzing H3K4 methylation in *Drosophila* embryos indicates that DV enhancers acquire H3K4me1 at ZGA in both active (with H3K27ac) and uninduced (without H3K27ac) states, while both H3K4me1 and H3K4me3 are present at DV promoters (Koenecke *et al.*, 2017; Li *et al.*, 2014). It



would be interesting to apply the DV model system to distinguish cell type-specific differences in the deposition of H3K4 methylation and functionally interrogate the relationship between tissue-specific chromatin features and pause release during development.

### Transcription occurs in bursts that are modulated during developmental gene regulation

Transcription occurs in stochastic bursts of RNA synthesis, resulting from alternation between active and inactive states (Fig. 6) (Leyes Porello *et al.*, 2023; Rodriguez and Larson, 2020; Tunnacliffe and Chubb, 2020). The discontinuous nature of transcription was first observed in *Drosophila* embryo chromosome spreads by electron microscopy in the late 1970s (Miller and McKnight, 1979). Since then, the ability to study gene bursting has been spurred by the development of highly sensitive microscopy and genomics techniques that have been leveraged to explore transcriptional dynamics at the single-cell scale. *Drosophila* provides a major model system for studying gene bursting *in vivo* during development. Bursting has been visualized for genes of interest in early *Drosophila* embryos by single-molecule RNA FISH (smFISH) and captured live in real-time using the MS2/MCP system (Leyes Porello *et al.*, 2023). Gene bursting dynamics have also been inferred transcriptome-wide from single-cell RNA-seq (scRNA-seq) data (Hunt *et al.*, 2024; Jiang *et al.*, 2017; Kim and Marioni, 2013; Larsson *et al.*, 2019).

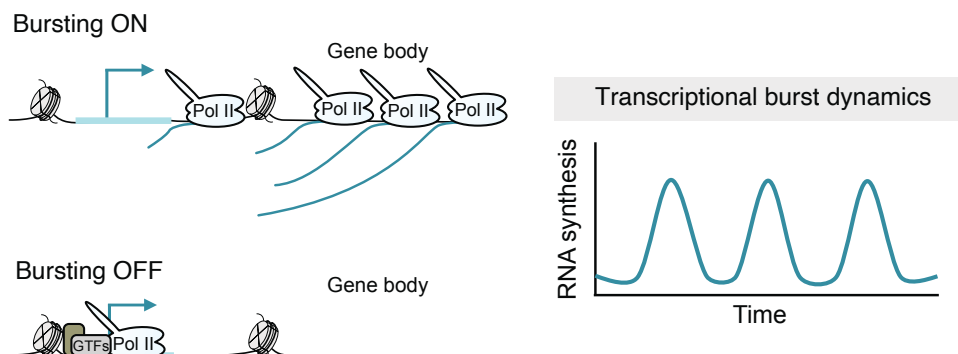
Several models, differing in the number of promoter activity states accommodated, have been applied to describe transcriptional bursts (Rodriguez and Larson, 2020; Tunnacliffe and Chubb, 2020). A two-state model, where promoters fluctuate between active/ON and inactive/OFF configurations, has been widely adopted to infer transcriptional dynamics (Berrocal *et al.*, 2020; Larsson *et al.*, 2019; Peccoud and Ycart, 1995; Raj *et al.*, 2006; Zoller *et al.*, 2018). Bursts have been characterized by parameters including frequency and size (number of RNA molecules produced per burst) that can be inferred from this model. However, the failure to accommodate intermediary promoter activity states, such as pausing, means the two-state model may not always satisfactorily capture promoter dynamics (Tunnacliffe and Chubb, 2020). As a result, multistate models have been proposed to better explain the transcriptional activity of some genes (Bartman *et al.*, 2019; Corrigan *et al.*, 2016; Neuert *et al.*, 2013; Pimmitt *et al.*, 2021; Suter *et al.*, 2011). While these experimental techniques and modelling approaches have

helped establish that gene burstiness is a highly evolutionarily conserved feature of transcription, a major focus has been to reveal the molecular determinants that modulate bursting dynamics.

Studies of the molecular determinants of bursts have linked enhancers to transcriptional control through modulation of gene burst frequencies (Fukaya *et al.*, 2016; Walters *et al.*, 1995). Chromatin features, such as differential histone acetylation at enhancers, have been found to correlate with the burst frequency of target genes (Larsson *et al.*, 2019; Nicolas *et al.*, 2018). The burst size of genes shows a strong association with the occurrence of specific core promoter motifs, suggesting that it is largely sequence-encoded at promoters (Hornung *et al.*, 2012; Larsson *et al.*, 2019). Inferring burst kinetics in *Drosophila* embryonic DV tissue scRNA-seq data corroborates that enhancer-mediated control of burst frequencies and promoter-encoded burst sizes underlie developmental gene induction *in vivo* (Hunt *et al.*, 2024). DV genes are characterized by a low burst frequency and the capacity for strong bursts. The high burst size capacity may derive from an overrepresentation of the core promoter motifs TATA and INR that bind TFIID to trigger PIC assembly (Haberle and Stark, 2018; Joo *et al.*, 2017), but could possibly also be influenced by pausing (Hunt *et al.*, 2024; Pimmitt *et al.*, 2021; Tantale *et al.*, 2021). Notably, differential promoter loading of P-TEFb between DV tissues correlates with burst size, implicating the pause release circuitry in the induction of strong bursts (Hunt *et al.*, 2024). The tissue-specific active chromatin state found at DV enhancers correlates strongly with elevated burst frequencies at target promoters but is also well correlated with burst size increases for genes whose induction does not involve significant changes in burst frequency. While the molecular determinants that modulate bursts are being revealed, much remains unclear about the mechanism of enhancer-promoter communication that allows burst induction to be signaled.

### Enhancer-promoter communication across the chromatin landscape

Enhancer-promoter communications occur in complex regulatory environments where they must overcome the challenges of genomic distance and specificity when surrounded by non-target enhancers and promoters. These communications occur within the genomic organization of TADs (Dixon *et al.*, 2012; Sexton *et al.*, 2012), with boundaries harboring insulator activities that prevent unintended



**Fig. 6. Gene induction often occurs through bursts of transcription.** Schematic of a gene in bursting ON and OFF configurations and a depiction of waves of RNA synthesis resulting from alternations between the ON and OFF states over time.

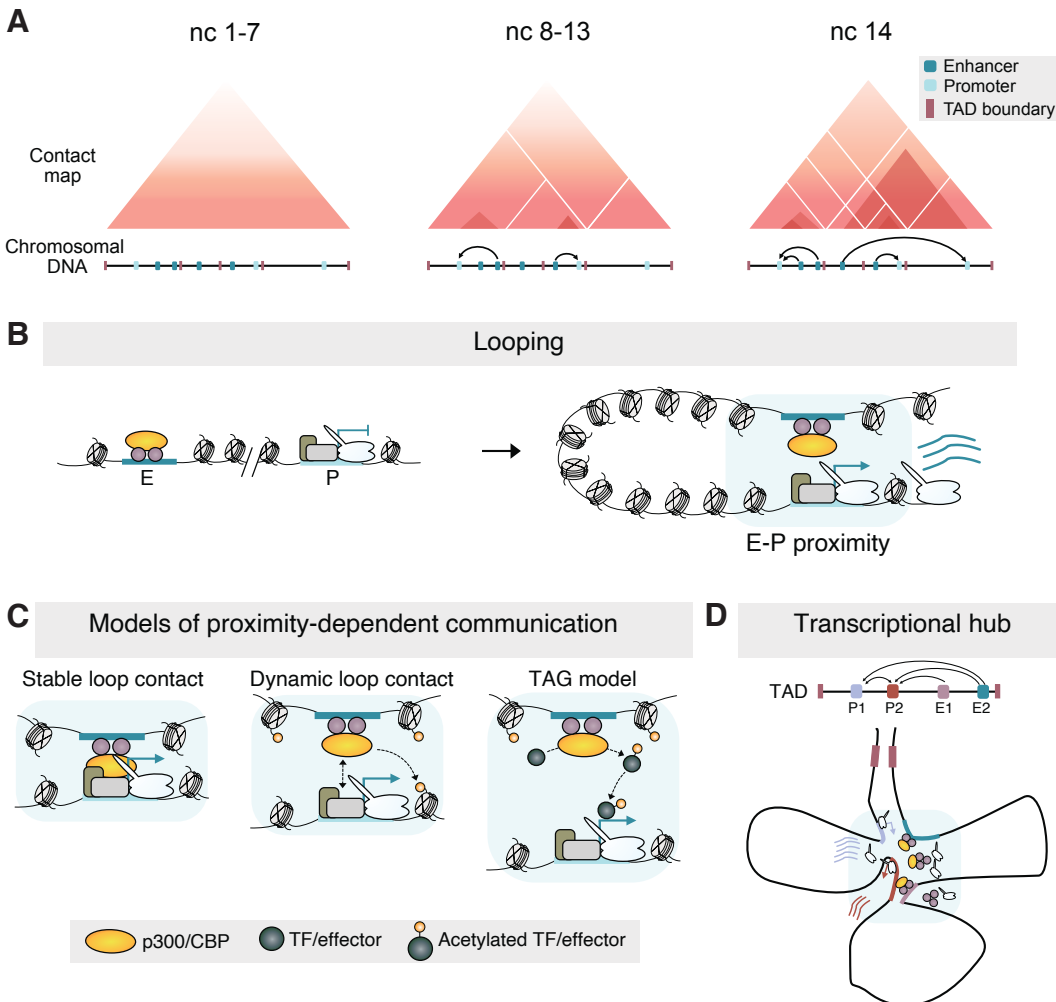
interactions between enhancers and promoters in different TADs (Jerkovic and Cavalli, 2021). Similar to their mammalian counterparts, *Drosophila* TAD boundaries are enriched in DNA binding motifs for insulator proteins (Hou *et al.*, 2012; Hug *et al.*, 2017; Jiang *et al.*, 2009; Kaushal *et al.*, 2022; Sexton *et al.*, 2012; Stadler *et al.*, 2017; Ulianov *et al.*, 2016). Techniques that capture chromosome conformation have revealed the emergence of *Drosophila* TADs and focal contacts between enhancers and promoters in parallel with ZGA (Fig. 7A) (Ghavi-Helm *et al.*, 2014; Hug *et al.*, 2017; Ogiyama *et al.*, 2018). In addition to containing motifs for insulator proteins, early forming

*Drosophila* TAD boundaries have specific signatures of histone modifications and can be enriched for Pol II and Zld (Hug *et al.*, 2017). TADs form independently of zygotic transcription, but insulation at some boundaries is compromised by transcriptional inhibition and depletion of Zld.

In mammals and *Drosophila*, there is disparity between the loss of TAD insulation when insulator proteins are perturbed and the modest effects on ensuing gene expression (Cavalheiro *et al.*, 2023; Kaushal *et al.*, 2021; Nora *et al.*, 2017; Rao *et al.*, 2017; Schwarzer *et al.*, 2017). Disruption of TAD boundaries in some instances have been demonstrated to rewire genome organization, leading to ectopic enhancer-promoter interactions that impact gene expression in mammals (Franke *et al.*, 2016; Lupiáñez *et al.*, 2015; Nora *et al.*, 2012). However, the depletion of architectural proteins understood to mediate contacts and the dramatically rearranged genome organization in *Drosophila* balancer chromosomes do not majorly affect enhancer-promoter communications and transcription (Ghavi-Helm *et al.*, 2019; Hsieh *et al.*, 2022). While TADs are reported to be largely invariant between cell types (Dixon *et al.*, 2015; Dixon *et al.*, 2012; Nora *et al.*, 2012), cell type-specific TADs have been detected, and intra-TAD interactions are reported to be dynamically regulated (Bonev *et al.*, 2017; Chathoth and Zabet,

2019; Kragestein *et al.*, 2018; Le Dily *et al.*, 2014; Mateo *et al.*, 2019). A class of CREs known as tethering elements has been shown to facilitate intra-TAD enhancer-promoter and promoter-promoter interactions in *Drosophila* (Batut *et al.*, 2022; Calhoun *et al.*, 2002). Moreover, GAF-mediated pairing of tethering elements may help ensure CRE selectivity (Batut *et al.*, 2022; Li *et al.*, 2023). Promoter-promoter contacts between promoter-proximal tethering elements have been shown to underlie the transcriptional coupling of genomically distant functionally-related genes with similar spatio-temporal expression dynamics driven by shared enhancers (Levo *et al.*, 2022). Some interactions between enhancers and promoters also occur across TADs (Batut *et al.*, 2022; Balasubramanian *et al.*, 2024; Yokoshi *et al.*, 2020).

While TADs are proposed to produce insulated genomic domains within which enhancers and promoters interact, the mechanisms behind these interactions remain unclear (Furlong and Levine, 2018). Chromatin looping is proposed to bring genomically distant enhancers and promoters into contact (Fig. 7B). The formation of chromatin loops may involve physical interactions between enhancer and promoter-bound architectural proteins, such as CTCF, and TFs with stable or transient proximity required for transcriptional activation (Fig. 7C). Both population-



**Fig. 7. 3D genome organization and models of enhancer-promoter communication.** (A) Schematic of genome organization during early *Drosophila* embryogenesis, showing the formation of TADs and the establishment of enhancer-promoter contacts. (B) Model presenting the looping topological conformation that brings enhancers and promoters into proximity. (C) Models illustrating proximity-dependent enhancer-promoter communication, showing stable and transient direct enhancer-promoter contacts mediated by physical interactions between enhancer- and promoter-bound factors. Dynamic interactions may transmit signals to promoters via the deposition of histone PTMs at promoter chromatin by enhancer-bound chromatin factors. The transcription factor activity gradient (TAG) model suggests an enhancer-localized activation signal, such as p300/CBP-mediated acetylation of TFs or transcriptional effector like P-TEFb, diffuses to the target promoter in close proximity to induce transcription (Karr *et al.*, 2022). (D) The transcriptional hub model for gene activation proposes the clustering of multiple enhancers and promoters in proximity. Within this hub environment, a pool of high concentrations of TFs, cofactors, and Pol II collectively drives robust activation (Furlong and Levine, 2018).

based chromosome conformation methods and imaging have observed enhancers and promoters in close physical proximity (Cruz-Molina *et al.*, 2017; Espinola *et al.*, 2021; Ghavi-Helm *et al.*, 2014; Hsieh *et al.*, 2020; Ing-Simmons *et al.*, 2021; Jin *et al.*, 2013; Krietenstein *et al.*, 2020; Li *et al.*, 2012; Sanyal *et al.*, 2012). Generally, enhancer-promoter proximity correlates with gene activity (Bonev *et al.*, 2017; Ghavi-Helm *et al.*, 2014; Mateo *et al.*, 2019; Rao *et al.*, 2014; Sanyal *et al.*, 2012), and forced looping can induce transcription of some genes (Bartman *et al.*, 2016; Deng *et al.*, 2012; Deng *et al.*, 2014). However, certain studies have detected no correlation, or even an anti-correlation, between enhancer-promoter proximity and gene activity (Alexander *et al.*, 2019; Benabdallah *et al.*, 2019; Chen *et al.*, 2018; Heist *et al.*, 2019). The model of dedicated and stable looping between an enhancer and its target promoter is challenged by the capacity of an enhancer to simultaneously drive bursts of transcription from flanking genes and to co-activate reporter genes *in cis* and *in trans* through the phenomenon of transvection in *Drosophila* embryos (Fukaya *et al.*, 2016; Lim *et al.*, 2018).

An alternative model of enhancer-promoter communication, not reliant on direct looping, is the TF activity gradient (TAG), proposing that acetylated TFs produced by enhancer-localized enzymatic p300/CBP activity diffuse to target promoters in close proximity to activate transcription (Fig. 7C) (Karr *et al.*, 2022). In this model, histone deacetylases (HDACs), chromatin modifiers that also enzymatically target non-histone proteins (Glozak *et al.*, 2005), deacetylate TFs to spatially limit the diffusing acetylated TF signal. HDACs are recruited to active promoters in metazoans, where they may attenuate acetylated TF signals (Filion *et al.*, 2010; Rincon-Arango *et al.*, 2012; Wang *et al.*, 2008). The quantity of molecules from coactivators such as p300/CBP appears to be limited in cells, being far surpassed by HDACs (Gillespie *et al.*, 2020). Consistently, pharmacological HDAC inhibition in *Drosophila* cells rapidly elevates histone acetylation levels at active promoters and correlates with Pol II promoter release (Vaid *et al.*, 2020). Overall, the relationship between enhancer-promoter proximity and the transfer of regulatory cues that lead to activation remains unresolved.

### Gene activation in invariant chromosome conformations

During *Drosophila* embryogenesis, enhancer-promoter proximities are established before transcriptional activation (Espinola *et al.*, 2021; Ghavi-Helm *et al.*, 2014). Strikingly, despite substantial differences in chromatin state and transcriptional activity, DV loci do not markedly change in chromatin conformation between DV tissues (Ing-Simmons *et al.*, 2021). High-resolution imaging of CRE interactions within the dorsocross (doc) TAD, which encompasses three co-expressed dorsal ectoderm-specific genes, revealed that multiple enhancers and promoters coalesce in close proximity independently of cell fate (Espinola *et al.*, 2021). Similarly, anterior-posterior regulated genes display a consistent chromatin conformation in both anterior and posterior halves of early *Drosophila* embryos, despite their distinct transcriptional states (Stadler *et al.*, 2017). These observations suggest that a preformed topology with close proximity between enhancers and promoters might be a crucial prerequisite for gene activity, but additional signals are necessary to initiate transcription.

Interestingly, promoters associated with preformed interactions are enriched for paused Pol II, further implying their priming for activity in response to the required regulatory cue (Ghavi-Helm *et al.*, 2014).

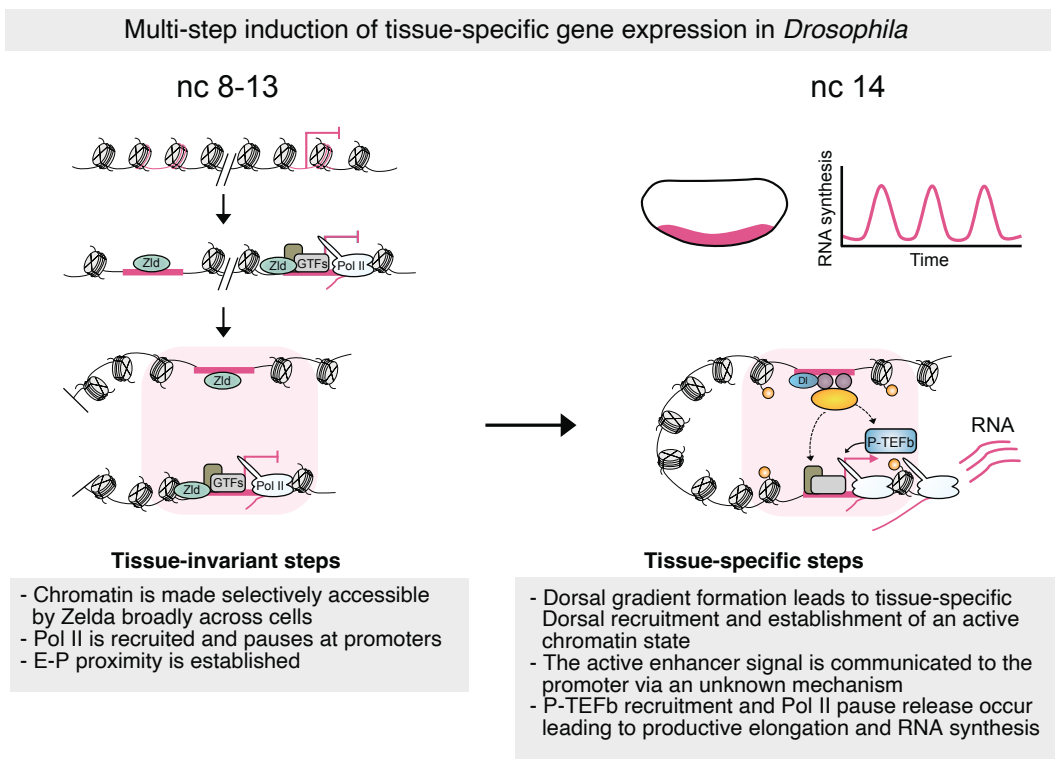
### Nuclear hubs and phase separation in gene activation

Observations of close proximity between potentially multiple enhancers and promoters are consistent with the concept of nuclear hubs or transcription factories, where high concentrations of TFs, cofactors, and the Pol II machinery drive robust bursts of gene expression (Fig. 7D) (Furlong and Levine, 2018; Iborra *et al.*, 1996; Lim and Levine, 2021). Zelda forms sub-nuclear hubs of high concentration within which frequent transient binding events occur that potentiate gene expression during *Drosophila* embryonic patterning (Dufourt *et al.*, 2018; Mir *et al.*, 2018). Notably, the size of enhancer TF clusters of the anterior-posterior patterning morphogen Bicoid correlates with the burst size of target reporter genes (Kawasaki and Fukaya, 2023).

The process of liquid-liquid phase separation (LLPS), where dense cooperative assemblies of proteins form liquid-like condensates or droplets, has been proposed to explain how high sub-nuclear concentrations of transcriptional components might be achieved (Hnisz *et al.*, 2017). Condensate formation is thought to be driven by multivalent interactions through intrinsically disordered regions (IDRs). Liquid-like condensate formation has been associated with the transcriptional capacity of several coactivators (Boija *et al.*, 2018; Cho *et al.*, 2018; Sabari *et al.*, 2018) and Pol II (Boehning *et al.*, 2018; Cho *et al.*, 2018; Guo *et al.*, 2019; Lu *et al.*, 2018), which have consistently been detected in nuclear clusters by various methods (Cisse *et al.*, 2013; Cook, 1999; Pownall *et al.*, 2023). p300/CBP nuclear condensates are linked to enhanced HAT activity at active genomic sites (Ma *et al.*, 2021) but also the sequestration of HAT inactive condensates in H3K27me3-enriched chromatin (Zhang *et al.*, 2021).

Condensates are also influenced by DNA sequence, with enhancer elements dense in TF recruitment motifs supporting condensate formation *in vitro* (Shrinivas *et al.*, 2019). While IDR interactions may underlie TF and Pol II clustering (Chong *et al.*, 2018), much debate concerns whether cluster formation *in vivo* is attributed to LLPS and what relevance this may pertain to transcription (Alberti *et al.*, 2019; McSwiggen *et al.*, 2019). Multivalent clustering has been shown to enhance the transcription activation strength of synthetic TFs, but liquid-like droplet formation did not (Trojanowski *et al.*, 2022).

The formation of localized clusters of TFs, cofactors and the Pol II machinery into hubs at enhancers and promoters in close proximity presents a plausible organization for gene regulatory circuitry. Interestingly, RNAs associated with transcription initiation stimulate condensate formation, while RNAs produced during bursts of elongation have been linked to condensate dissociation (Henninger *et al.*, 2021). This observation aligns with Pol II cluster dynamics observed through chromatin expansion microscopy, where enhancer-promoter interactions occur transiently, and bursts of transcriptional elongation are associated with their separation, termed the "kiss and kick" model (Pownall *et al.*, 2023). At present, what constitutes the transmissible enhancer signal is unclear.



**Fig. 8. Developmental gene induction during *Drosophila* developmental patterning.** The current understanding of *Drosophila* DV gene regulation suggests a multi-step process in the activation of DV-regulated genes. Chromatin is made accessible by Zelda at key enhancers and promoters. Following this, Pol II pausing is established, and enhancer-promoter proximities form across cells prior to ZGA (Hunt *et al.*, 2024). Tissue-specific signals, in the form of the Dorsal (D) TF gradient and other tissue-specific TFs, lead to the formation of an active chromatin state. The communication of this signal to target promoters occurs via an unknown mechanism, ultimately inducing the release of paused Pol II from target promoters.

## Conclusion

*Drosophila* embryogenesis has served as a key model system for functionally dissecting developmental gene regulation. The rapid induction of cell type-specific transcriptional programs at ZGA well positions the *Drosophila* embryo for studying the molecular mechanisms governing how enhancers communicate regulatory cues to promoters and induce transcription. Leading up to ZGA, an elaborate genome organization forms with differential chromatin accessibility, TF binding, and histone PTM deposition, along with the establishment of proximity between enhancers and promoters. The interaction of these different layers of genome regulation to direct transcription across space and time remains unresolved. A major goal is to obtain a mechanistic understanding of how regulatory information is transferred between enhancers and promoters and what the communicable signal may be. It is striking that chromosome conformation is similar in different DV cell types, despite their major differences in chromatin and transcriptional states. This suggests a preformed topology may be a prerequisite, but not the molecular trigger, of enhancer-promoter communication. Such an organization could facilitate multi-step regulation of developmental transcription, as seen in the DV GRN. Here, within a pre-established organizational architecture of enhancer-promoter proximity, TFs and cofactors may cluster at enhancers in a cell type-specific manner to initiate bursts of transcription from promoters primed with paused Pol II (Fig. 8). The DV GRN is well-positioned for future efforts to functionally interrogate potential mediators of communication, such as rapid optogenetic depletion of p300/CBP or effectors it may modify, like BRD4 or P-TEFb, as achieved in the early embryo for other factors (Huang *et al.*, 2017; McDaniel *et al.*, 2019; Singh *et al.*, 2022).

A key aspect of elucidating enhancer-promoter communication is pinpointing the steps in the transcriptional cycle where regulatory influence is integrated. The DV GRN suggests that tissue-specific transcription is driven by post-recruitment control of the transition of Pol II from pausing to elongating. The recruitment of paused Pol II across cells in the early embryo suggests the widespread adoption of a promoter state conducive to tissue-specific signals. Enhancer-driven transcriptional induction, achieved through control of pause release, is consistent with modulation of the pause release machinery being a key output of enhancer activation. *Drosophila* embryogenesis is primed to continue to play a pivotal role in unravelling these unknowns.

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## Conflicts of interest

The authors declare that they have no conflicts of interest.

## References

- ALBERTI S., GLADFELTER A., MITTAG T. (2019). Considerations and Challenges in Studying Liquid-Liquid Phase Separation and Biomolecular Condensates. *Cell* 176: 419-434. <https://doi.org/10.1016/j.cell.2018.12.035>
- ALEXANDER J. M., GUAN J., LI B., MALISOVA L., SONG M., SHEN Y., HUANG B., LOMVARDAS S., WEINER O. D. (2019). Live-cell imaging reveals enhancer-dependent Sox2 transcription in the absence of enhancer proximity. *eLife* 8: e41769. <https://doi.org/10.7554/eLife.41769>
- ALLFREY V. G., FAULKNER R., MIRSKY A. E. (1964). ACETYLATION AND METHYLATION OF HISTONES AND THEIR POSSIBLE ROLE IN THE REGULATION OF RNA SYNTHESIS. *Proceedings of the National Academy of Sciences* 51: 786-794. <https://doi.org/10.1073/pnas.51.5.786>

- ASHE H. L., MANNERVIK M., LEVINE M. (2000). Dpp signaling thresholds in the dorsal ectoderm of the *Drosophila* embryo. *Development* 127: 3305-3312. <https://doi.org/10.1242/dev.127.15.3305>
- BALASUBRAMANIAN D., BORGES PINTO P., GRASSO A., VINCENT S., TARAYRE H., LAJOIGNIE D., GHAVI-HELM Y. (2024). Enhancer-promoter interactions can form independently of genomic distance and be functional across TAD boundaries. *Nucleic Acids Research* 52: 1702-1719. <https://doi.org/10.1093/nar/gkad1183>
- BALSALOBRE A., DROUIN J. (2022). Pioneer factors as master regulators of the epigenome and cell fate. *Nature Reviews Molecular Cell Biology* 23: 449-464. <https://doi.org/10.1038/s41580-022-00464-z>
- BANERJI J., OLSON L., SCHAFFNER W. (1983). A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. *Cell* 33: 729-740. [https://doi.org/10.1016/0092-8674\(83\)90015-6](https://doi.org/10.1016/0092-8674(83)90015-6)
- BANERJI J., RUSCONI S., SCHAFFNER W. (1981). Expression of a  $\beta$ -globin gene is enhanced by remote SV40 DNA sequences. *Cell* 27: 299-308. [https://doi.org/10.1016/0092-8674\(81\)90413-X](https://doi.org/10.1016/0092-8674(81)90413-X)
- BANNISTER A. J., KOUZARIDES T. (2011). Regulation of chromatin by histone modifications. *Cell Research* 21: 381-395. <https://doi.org/10.1038/cr.2011.22>
- BARBORIC M., NISSEN R. M., KANAZAWA S., JABRANE-FERRAT N., PETERLIN B.M. (2001). NF- $\kappa$ B Binds P-TEFb to Stimulate Transcriptional Elongation by RNA Polymerase II. *Molecular Cell* 8: 327-337. [https://doi.org/10.1016/S1097-2765\(01\)00314-8](https://doi.org/10.1016/S1097-2765(01)00314-8)
- BARTMAN C. R., HAMAGAMI N., KELLER C. A., GIARDINE B., HARDISON R. C., BLOBEL G. A., RAJ A. (2019). Transcriptional Burst Initiation and Polymerase Pause Release Are Key Control Points of Transcriptional Regulation. *Molecular Cell* 73: 519-532.e4. <https://doi.org/10.1016/j.molcel.2018.11.004>
- BARTMAN C. R., HSU S. C., HSIUNG C. C.S., RAJ A., BLOBEL G. A. (2016). Enhancer Regulation of Transcriptional Bursting Parameters Revealed by Forced Chromatin Looping. *Molecular Cell* 62: 237-247. <https://doi.org/10.1016/j.molcel.2016.03.007>
- BATUT P. J., BING X. Y., SISCO Z., RAIMUNDO J., LEVO M., LEVINE M. S. (2022). Genome organization controls transcriptional dynamics during development. *Science* 375: 566-570. <https://doi.org/10.1126/science.abi7178>
- BEDFORD D. C., BRINDLE P. K. (2012). Is histone acetylation the most important physiological function for CBP and p300?. *Aging* 4: 247-255. <https://doi.org/10.18632/aging.100453>
- BELLE C. M., DUFOUR J., HUNT G., LENDEN-HASSE H., TRULLO A., ZINE EL AABIDINE A., LAMARQUE M., GASKILL M. M., FAURE-GAUTRON H., MANNERVIK M., HARRISON M. M., ANDRAU J.C., et al. (2022). The control of transcriptional memory by stable mitotic bookmarking. *Nature Communications* 13: 1176. <https://doi.org/10.1038/s41467-022-28855-y>
- BELVIN M. P., JIN Y., ANDERSON K. V. (1995). Cactus protein degradation mediates *Drosophila* dorsal-ventral signaling. *Genes & Development* 9: 783-793. <https://doi.org/10.1101/gad.9.7.783>
- BENABDALLAH N. S., WILLIAMSON I., ILLINGWORTH R. S., KANE L., BOYLE S., SENGUPTA D., GRIMES G. R., THERIZOL S.P., BICKMORE W. A. (2019). Decreased Enhancer-Promoter Proximity Accompanying Enhancer Activation. *Molecular Cell* 76: 473-484.e7. <https://doi.org/10.1016/j.molcel.2019.07.038>
- BERNSTEIN B. E., MIKKELSEN T. S., XIE X., KAMAL M., HUEBERT D. J., CUFF J., FRY B., MEISSNER A., WERNIG M., PLATH K., JAENISCH R., WAGSCHAL A., et al. (2006). A Bivalent Chromatin Structure Marks Key Developmental Genes in Embryonic Stem Cells. *Cell* 125: 315-326. <https://doi.org/10.1016/j.cell.2006.02.041>
- BERROCAL A., LAMMERS N. C., GARCIA H. G., EISEN M. B. (2020). Kinetic sculpting of the seven stripes of the *Drosophila* even-skipped gene. *eLife* 9: e61635. <https://doi.org/10.7554/eLife.61635>
- BIEMAR F., NIX D. A., PIEL J., PETERSON B., RONSHAUGEN M., SEMENTCHENKO V., BELL I., MANAK J. R., LEVINE M. S. (2006). Comprehensive identification of *Drosophila* dorsal-ventral patterning genes using a whole-genome tiling array. *Proceedings of the National Academy of Sciences* 103: 12763-12768. <https://doi.org/10.1073/pnas.0604484103>
- BLYTHES A. S., WIESCHAUS E. F. (2016). Establishment and maintenance of heritable chromatin structure during early *Drosophila* embryogenesis. *eLife* 5: e20148. <https://doi.org/10.7554/eLife.20148>
- BOEHNING M., DUGAST-DARZACQ C., RANKOVIC M., HANSEN A. S., YU T., MARIELLY H., MCSWIGGEN D. T., KOKIC G., DAILEY G. M., CRAMER P., DARZACQ X., ZWECKSTETTER M. (2018). RNA polymerase II clustering through carboxy-terminal domain phase separation. *Nature Structural & Molecular Biology* 25: 833-840. <https://doi.org/10.1038/s41594-018-0112-y>
- BOETTIGER A. N., LEVINE M. (2009). Synchronous and Stochastic Patterns of Gene Activation in the *Drosophila* Embryo. *Science* 325: 471-473. <https://doi.org/10.1126/science.1173976>
- BOGDANOVIĆ O., FERNANDEZ-MIÑÁN A., TENA J. J., DE LA CALLE-MUSTIENES E., HIDALGO C., VAN KRUYSSBERGEN I., VAN HEERINGEN S. J., VEENSTRA G. J. C., GÓMEZ-SKARMETA J. L. (2012). Dynamics of enhancer chromatin signatures mark the transition from pluripotency to cell specification during embryogenesis. *Genome Research* 22: 2043-2053. <https://doi.org/10.1101/gr.134833.111>
- BOIJA A., KLEIN I. A., SABARI B. R., DALL'AGNESE A., COFFEY E. L., ZAMUDIO A. V., LI C. H., SHRINIVAS K., MANTEIGA J. C., HANNETT N. M., ABRAHAM B. J., AFEYAN L. K., et al. (2018). Transcription Factors Activate Genes through the Phase-Separation Capacity of Their Activation Domains. *Cell* 175: 1842-1855.e16. <https://doi.org/10.1016/j.cell.2018.10.042>
- BOIJA A., MAHAT D. B., ZARE A., HOLMQVIST P.H., PHILIP P., MEYERS D. J., COLE P. A., LIS J. T., STENBERG P., MANNERVIK M. (2017). CBP Regulates Recruitment and Release of Promoter-Proximal RNA Polymerase II. *Molecular Cell* 68: 491-503.e5. <https://doi.org/10.1016/j.molcel.2017.09.031>
- BOIJA A., MANNERVIK M. (2016). Initiation of diverse epigenetic states during nuclear programming of the *Drosophila* body plan. *Proceedings of the National Academy of Sciences* 113: 8735-8740. <https://doi.org/10.1073/pnas.1516450113>
- BONEV B., MENDELSON COHEN N., SZABO Q., FRITSCH L., PAPADOPOULOS G. L., LUBLING Y., XU X., LV X., HUGNOT J.P., TANAY A., CAVALLI G. (2017). Multiscale 3D Genome Rewiring during Mouse Neural Development. *Cell* 171: 557-572.e24. <https://doi.org/10.1016/j.cell.2017.09.043>
- BONN S., ZINZEN R. P., GIRARDOT C., GUSTAFSON E. H., PEREZ-GONZALEZ A., DELHOMME N., GHAVI-HELM Y., WILCZYŃSKI B., RIDDELL A., FURLONG E. E. M. (2012). Tissue-specific analysis of chromatin state identifies temporal signatures of enhancer activity during embryonic development. *Nature Genetics* 44: 148-156. <https://doi.org/10.1038/ng.1064>
- BOSE D. A., DONAHUE G., REINBERG D., SHIEKHATTARR, BONASIOR, BERGERS L. (2017). RNA Binding to CBP Stimulates Histone Acetylation and Transcription. *Cell* 168: 135-149.e22. <https://doi.org/10.1016/j.cell.2016.12.020>
- BOYLE A. P., DAVIS S., SHULHA H. P., MELTZER P., MARGULIES E. H., WENG Z., FUREY T. S., CRAWFORD G. E. (2008). High-Resolution Mapping and Characterization of Open Chromatin across the Genome. *Cell* 132: 311-322. <https://doi.org/10.1016/j.cell.2007.12.014>
- BOZEK M., CORTINI R., STORTI A. E., UNNERSTALL U., GAUL U., GOMPEL N. (2019). ATAC-seq reveals regional differences in enhancer accessibility during the establishment of spatial coordinates in the *Drosophila* blastoderm. *Genome Research* 29: 771-783. <https://doi.org/10.1101/gr.242362.118>
- CAIRNS B. R. (2009). The logic of chromatin architecture and remodelling at promoters. *Nature* 461: 193-198. <https://doi.org/10.1038/nature08450>
- CALDERON D., BLECHER-GONEN R., HUANG X., SECCHIA S., KENTRO J., DAZA R. M., MARTIN B., DULJA A., SCHAUB C., TRAPNELL C., LARSCHAN E., O'CONNOR-GILES K. M., et al. (2022). The continuum of *Drosophila* embryonic development at single-cell resolution. *Science* 377: eabn5800. <https://doi.org/10.1126/science.abn5800>
- CALHOUN V. C., STATHOPOULOS A., LEVINEM. (2002). Promoter-proximal tethering elements regulate enhancer-promoter specificity in the *Drosophila* Antennapedia complex. *Proceedings of the National Academy of Sciences* 99: 9243-9247. <https://doi.org/10.1073/pnas.142291299>
- CANNAVÒ E., KHOUEIRY P., GARFIELD D. A., GEELEHER P., ZICHNERT, GUSTAFSON E. H., CIGLAR L., KORBEL J. O., FURLONG E. E. M. (2016). Shadow Enhancers Are Pervasive Features of Developmental Regulatory Networks. *Current Biology* 26: 38-51. <https://doi.org/10.1016/j.cub.2015.11.034>
- CATARINO R. R., STARK A. (2018). Assessing sufficiency and necessity of enhancer activities for gene expression and the mechanisms of transcription activation. *Genes & Development* 32: 202-223. <https://doi.org/10.1101/gad.310367.117>
- CAVALHEIRO G. R., GIRARDOT C., VIALES R. R., POLLEX T., CAO T. B. N., LACOUR P., FENG S., RABINOWITZ A., FURLONG E. E. M. (2023). CTCF, BEAF-32, and CP190 are not required for the establishment of TADs in early *Drosophila* embryos but have locus-specific roles. *Science Advances* 9: ead1085. <https://doi.org/10.1126/sciadv.ade1085>
- CHAN H. M., LA THANGUE N. B. (2001). p300/CBP proteins: HATs for transcriptional bridges and scaffolds. *Journal of Cell Science* 114: 2363-2373. <https://doi.org/10.1242/jcs.114.13.2363>

- CHAN S. H., TANG Y., MIAO L., DARWICH-CODORE H., VEJNAR C. E., BEAUDOIN J. D., MUSAIEV D., FERNANDEZ J. P., BENITEZ M. D. J., BAZZINI A. A., MORENO-MATEOS M. A., GIRALDEZ A. J. (2019). Brd4 and P300 Confer Transcriptional Competency during Zygotic Genome Activation. *Developmental Cell* 49: 867-881. e8. <https://doi.org/10.1016/j.devcel.2019.05.037>
- CHATHOTH K. T., ZABET N. R. (2019). Chromatin architecture reorganization during neuronal cell differentiation in Drosophila genome. *Genome Research* 29: 613-625. <https://doi.org/10.1101/gr.246710.118>
- CHEN H., LEVO M., BARINOV L., FUJIOKA M., JAYNES J. B., GREGOR T. (2018). Dynamic interplay between enhancer-promoter topology and gene activity. *Nature Genetics* 50: 1296-1303. <https://doi.org/10.1038/s41588-018-0175-z>
- CHEN K., JOHNSTON J., SHAO W., MEIER S., STABER C., ZEITLINGER J. (2013). A global change in RNA polymerase II pausing during the Drosophila midblastula transition. *eLife* 2: e00861. <https://doi.org/10.7554/eLife.00861>
- CHO H., ORPHANIDES G., SUN X., YANG X. J., OGRYZKO V., LEES E., NAKATANI Y., REINBERG D. (1998). A Human RNA Polymerase II Complex Containing Factors That Modify Chromatin Structure. *Molecular and Cellular Biology* 18: 5355-5363. <https://doi.org/10.1128/MCB.18.9.5355>
- CHOS., SCHROEDERS., KAEHLCKE K., KWON H. S., PEDALA., HERKERE., SCHNOELZER M., OTT M. (2009). Acetylation of cyclin T1 regulates the equilibrium between active and inactive P-TEFb in cells. *The EMBO Journal* 28: 1407-1417. <https://doi.org/10.1038/emboj.2009.99>
- CHO W. K., SPILLE J. H., HECHT M., LEE C., LI C., GRUBE V., CISSE I. I. (2018). Mediator and RNA polymerase II clusters associate in transcription-dependent condensates. *Science* 361: 412-415. <https://doi.org/10.1126/science.aar4199>
- CHONG S., DUGAST-DARZACQ C., LIU Z., DONG P., DAILEY G. M., CATTOGLIO C., HECKERT A., BANALA S., LAVIS L., DARZACQ X., TJIAN R. (2018). Imaging dynamic and selective low-complexity domain interactions that control gene transcription. *Science* 361: eaar2555. <https://doi.org/10.1126/science.aar2555>
- CIABRELLI F., RABBANI L., CARDAMONE F., ZENK F., LÖSER E., SCHÄCHTLE M. A., MAZINA M., LOUBIERE V., IOVINO N. (2023). CBP and Gcn5 drive zygotic genome activation independently of their catalytic activity. *Science Advances* 9: eadf2687. <https://doi.org/10.1126/sciadv.adf2687>
- CISSE I. I., IZEDDIN I., CAUSSE S. Z., BOUDARENE L., SENEAL A., MURESAN L., DUGAST-DARZACQ C., HAJJ B., DAHAN M., DARZACQ X. (2013). Real-Time Dynamics of RNA Polymerase II Clustering in Live Human Cells. *Science* 341: 664-667. <https://doi.org/10.1126/science.1239053>
- COOK P. R. (1999). The Organization of Replication and Transcription. *Science* 284: 1790-1795. <https://doi.org/10.1126/science.284.5421.1790>
- CORE L., ADELMAN K. (2019). Promoter-proximal pausing of RNA polymerase II: a nexus of gene regulation. *Genes & Development* 33: 960-982. <https://doi.org/10.1101/gad.325142.119>
- CORE L. J., WATERFALL J. J., LIS J. T. (2008). Nascent RNA Sequencing Reveals Widespread Pausing and Divergent Initiation at Human Promoters. *Science* 322: 1845-1848. <https://doi.org/10.1126/science.1162228>
- CORRIGAN A. M., TUNNAcliffe E., CANNON D., CHUBB J. R. (2016). A continuum model of transcriptional bursting. *eLife* 5: e13051. <https://doi.org/10.7554/eLife.13051>
- CREYGHTON M. P., CHENG A. W., WELSTEAD G. G., KOOISTRA T., CAREY B. W., STEINE E. J., HANNA J., LODATO M. A., FRAMPTON G. M., SHARP P. A., BOYER L. A., YOUNG R. A., et al. (2010). Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proceedings of the National Academy of Sciences* 107: 21931-21936. <https://doi.org/10.1073/pnas.1016071107>
- CRUZ-MOLINA S., RESPUELA P., TEBARTZ C., KOLOVOS P., NIKOLIC M., FUEYO R., VAN IJCKEN W. F. J., GROSVELD F., FROMMOLT P., BAZZI H., RADA-IGLESIAS A. (2017). PRC2 Facilitates the Regulatory Topology Required for Poised Enhancer Function during Pluripotent Stem Cell Differentiation. *Cell Stem Cell* 20: 689-705. e9. <https://doi.org/10.1016/j.stem.2017.02.004>
- CUSANOVICH D. A., REDDINGTON J. P., GARFIELD D. A., DAZAR M., AGHAMIRZAI D., MARCO-FERRERES R., PLINER H. A., CHRISTIANSEN L., QIU X., STEEMERS F. J., TRAPNELL C., SHENDURE J., et al. (2018). The cis-regulatory dynamics of embryonic development at single-cell resolution. *Nature* 555: 538-542. <https://doi.org/10.1038/nature25981>
- DAHL J. A., JUNG I., AANES H., GREGGAINS G. D., MANAF A., LERDRUP M., LI G., KUAN S., LI B., LEE A. Y., PREISSL S., JERMSTAD I., et al. (2016). Broad histone H3K4me3 domains in mouse oocytes modulate maternal-to-zygotic transition. *Nature* 537: 548-552. <https://doi.org/10.1038/nature19360>
- DANCY B. M., COLE P. A. (2015). Protein Lysine Acetylation by p300/CBP. *Chemical Reviews* 115: 2419-2452. <https://doi.org/10.1021/cr500452k>
- DANKO C. G., HAH N., LUO X., MARTINS A. L., CORE L., LIS J. T., SIEPEL A., KRAUS W. L. (2013). Signaling Pathways Differentially Affect RNA Polymerase II Initiation, Pausing, and Elongation Rate in Cells. *Molecular Cell* 50: 212-222. <https://doi.org/10.1016/j.molcel.2013.02.015>
- DAY D. S., ZHANG B., STEVENS S. M., FERRARI F., LARSCHAN E. N., PARK P. J., PU W. T. (2016). Comprehensive analysis of promoter-proximal RNA polymerase II pausing across mammalian cell types. *Genome Biology* 17: 120. <https://doi.org/10.1186/s13059-016-0984-2>
- DENG W., LEE J., WANG H., MILLER J., REIK A., GREGORY P. D., DEAN A., BLOBEL G. A. (2012). Controlling Long-Range Genomic Interactions at a Native Locus by Targeted Tethering of a Looping Factor. *Cell* 149: 1233-1244. <https://doi.org/10.1016/j.cell.2012.03.051>
- DENG W., RUPON J. W., KRIVEGA I., BREDA L., MOTTA I., JAHN K. S., REIK A., GREGORY P. D., RIVELLA S., DEAN A., BLOBEL G. A. (2014). Reactivation of Developmentally Silenced Globin Genes by Forced Chromatin Looping. *Cell* 158: 849-860. <https://doi.org/10.1016/j.cell.2014.05.050>
- DEY A., CHITSAZ F., ABBASI A., MISTELI T., OZATO K. (2003). The double bromodomain protein Brd4 binds to acetylated chromatin during interphase and mitosis. *Proceedings of the National Academy of Sciences* 100: 8758-8763. <https://doi.org/10.1073/pnas.1433065100>
- DIXON J. R., JUNG I., SELVARAJ S., SHEN Y., ANTOSIEWICZ-BOURGET J. E., LEE A. Y., YE Z., KIM A., RAJAGOPAL N., XIE W., DIAO Y., LIANG J., et al. (2015). Chromatin architecture reorganization during stem cell differentiation. *Nature* 518: 331-336. <https://doi.org/10.1038/nature14222>
- DIXON J. R., SELVARAJ S., YUE F., KIM A., LI Y., SHEN Y., HU M., LIU J. S., REN B. (2012). Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* 485: 376-380. <https://doi.org/10.1038/nature11082>
- DORIGHI K. M., SWIGUT T., HENRIQUES T., BHANU N. V., SCRUGGS B. S., NADY N., STILL C. D., GARCIA B. A., ADELMAN K., WYSOCKA J. (2017). Mll3 and Mll4 Facilitate Enhancer RNA Synthesis and Transcription from Promoters Independently of H3K4 Monomethylation. *Molecular Cell* 66: 568-576.e4. <https://doi.org/10.1016/j.molcel.2017.04.018>
- DUAN J., RIEDER L., COLONNETTA M. M., HUANG A., MCKENNEY M., WATTERS S., DESHPANDE G., JORDAN W., FAWZI N., LARSCHAN E. (2021). CLAMP and Zelda function together to promote Drosophila zygotic genome activation. *eLife* 10: e69937. <https://doi.org/10.7554/eLife.69937>
- DUBNICOFF T., VALENTINE S. A., CHEN G., SHI T., LENGUEL J. A., PAROUSH Z., COUREY A. J. (1997). Conversion of Dorsal from an activator to a repressor by the global corepressor Groucho. *Genes & Development* 11: 2952-2957. <https://doi.org/10.1101/gad.11.22.2952>
- DUFORT J., TRULLO A., HUNTER J., FERNANDEZ C., LAZARO J., DEJEAN M., MORALES L., NAIT-AMER S., SCHULZ K. N., HARRISON M. M., FAVARD C., RADULESCU O., et al. (2018). Temporal control of gene expression by the pioneer factor Zelda through transient interactions in hubs. *Nature Communications* 9: 5194. <https://doi.org/10.1038/s41467-018-07613-z>
- ENCODE PROJECT CONSORTIUM (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature* 489: 57-74. <https://doi.org/10.1038/nature11247>
- ENDERLE D., BEISEL C., STADLER M. B., GERSTUNG M., ATHRI P., PARO R. (2011). Polycomb preferentially targets stalled promoters of coding and noncoding transcripts. *Genome Research* 21: 216-226. <https://doi.org/10.1101/gr.114348.110>
- ESPINOLA S. M., GÖTZ M., BELLEC M., MESSINA O., FICHE J. B., HOUBRON C., DEJEAN M., REIM I., CARDOZO GIZZI A. M., LAGHA M., NOLLMANN M. (2021). Cis-regulatory chromatin loops arise before TADs and gene activation, and are independent of cell fate during early Drosophila development. *Nature Genetics* 53: 477-486. <https://doi.org/10.1038/s41588-021-00816-z>
- FELLER C., FORNÉ I., IMHOF A., BECKER P. B. (2015). Global and Specific Responses of the Histone Acetylome to Systematic Perturbation. *Molecular Cell* 57: 559-571. <https://doi.org/10.1016/j.molcel.2014.12.008>
- FENLEY A. T., ADAMS D. A., ONUFRIE V. A. V. (2010). Charge State of the Globular Histone Core Controls Stability of the Nucleosome. *Biophysical Journal* 99: 1577-1585. <https://doi.org/10.1016/j.bpj.2010.06.046>
- FERGUSON E. L., ANDERSON K. V. (1992). decapentaplegic acts as a morphogen to organize dorsal-ventral pattern in the Drosophila embryo. *Cell* 71: 451-461. [https://doi.org/10.1016/0092-8674\(92\)90514-D](https://doi.org/10.1016/0092-8674(92)90514-D)

- FIANU I., CHEN Y., DIENEMANN C., DYBKOV O., LINDEN A., URLAUB H., CRAMER P. (2021). Structural basis of Integrator-mediated transcription regulation. *Science* 374: 883-887. <https://doi.org/10.1126/science.abk0154>
- FILION G. J., VAN BEMMEL J. G., BRAUNSCHEWIG U., TALHOUT W., KIND J., WARD L. D., BRUGMAN W., DE CASTRO I. J., KERKHOVEN R. M., BUSSEMAKER H. J., VAN STEENSEL B. (2010). Systematic Protein Location Mapping Reveals Five Principal Chromatin Types in *Drosophila* Cells. *Cell* 143: 212-224. <https://doi.org/10.1016/j.cell.2010.09.009>
- FLAVAHAN W. A., GASKELL E., BERNSTEIN B. E. (2017). Epigenetic plasticity and the hallmarks of cancer. *Science* 357: aal2380. <https://doi.org/10.1126/science.aal2380>
- FRANKE M., IBRAHIM D. M., ANDREY G., SCHWARZER W., HEINRICH V., SCHÖPFLIN R., KRAFT K., KEMPFER R., JERKOVIĆ I., CHAN W. L., SPIELMANN M., TIMMERMANN B., et al. (2016). Formation of new chromatin domains determines pathogenicity of genomic duplications. *Nature* 538: 265-269. <https://doi.org/10.1038/nature19800>
- FRANKEL N., DAVIS G. K., VARGAS D., WANG S., PAYRE F., STERN D. L. (2010). Phenotypic robustness conferred by apparently redundant transcriptional enhancers. *Nature* 466: 490-493. <https://doi.org/10.1038/nature09158>
- FUJINAGA K., HUANG F., PETERLIN B. M. (2023). P-TEFb: The master regulator of transcription elongation. *Molecular Cell* 83: 393-403. <https://doi.org/10.1016/j.molcel.2022.12.006>
- FUJISAWA T., FILIPPAKOPOULOS P. (2017). Functions of bromodomain-containing proteins and their roles in homeostasis and cancer. *Nature Reviews Molecular Cell Biology* 18: 246-262. <https://doi.org/10.1038/nrm.2016.143>
- FUKAYA T., LIM B., LEVINE M. (2016). Enhancer Control of Transcriptional Bursting. *Cell* 166: 358-368. <https://doi.org/10.1016/j.cell.2016.05.025>
- FURLONG E. E. M., LEVINE M. (2018). Developmental enhancers and chromosome topology. *Science* 361: 1341-1345. <https://doi.org/10.1126/science.aau0320>
- GAERTNER B., JOHNSTON J., CHEN K., WALLASCHEK N., PAULSON A., GARRUSS A. S., GAUDENZ K., DE KUMAR B., KRUMLAUF R., ZEITLINGER J. (2012). Poised RNA Polymerase II Changes over Developmental Time and Prepares Genes for Future Expression. *Cell Reports* 2: 1670-1683. <https://doi.org/10.1016/j.celrep.2012.11.024>
- GAERTNER B., ZEITLINGER J. (2014). RNA polymerase II pausing during development. *Development* 141: 1179-1183. <https://doi.org/10.1242/dev.088492>
- GASKILL M. M., GIBSON T. J., LARSON E. D., HARRISON M. M. (2021). GAF is essential for zygotic genome activation and chromatin accessibility in the early *Drosophila* embryo. *eLife* 10: e66668. <https://doi.org/10.7554/eLife.66668>
- GHAVI-HELM Y., JANKOWSKI A., MEIERS S., VIALES R. R., KORBEL J. O., FURLONG E. E. M. (2019). Highly rearranged chromosomes reveal uncoupling between genome topology and gene expression. *Nature Genetics* 51: 1272-1282. <https://doi.org/10.1038/s41588-019-0462-3>
- GHAVI-HELM Y., KLEIN F. A., PAKOZDI T., CIGLAR L., NOORDERMEER D., HUBER W., FURLONG E. E. M. (2014). Enhancer loops appear stable during development and are associated with paused polymerase. *Nature* 512: 96-100. <https://doi.org/10.1038/nature13417>
- GILCHRIST D. A., DOS SANTOS G., FARGO D. C., XIE B., GAO Y., LI L., ADELMAN K. (2010). Pausing of RNA Polymerase II Disrupts DNA-Specified Nucleosome Organization to Enable Precise Gene Regulation. *Cell* 143: 540-551. <https://doi.org/10.1016/j.cell.2010.10.004>
- GILLESPIEM A., PALIIC G., SANCHEZ-TALTAVULLD., SHANNON P., LONGABAUGH W. J. R., DOWNES D. J., SIVARAMAN K., ESPINOZA H. M., HUGHES J. R., PRICE N. D., PERKINS T. J., RANISH J. A., et al. (2020). Absolute Quantification of Transcription Factors Reveals Principles of Gene Regulation in Erythropoiesis. *Molecular Cell* 78: 960-974.e11. <https://doi.org/10.1016/j.molcel.2020.03.031>
- GILLIES S. D., MORRISON S. L., OI V. T., TONEGAWA S. (1983). A tissue-specific transcription enhancer element is located in the major intron of a rearranged immunoglobulin heavy chain gene. *Cell* 33: 717-728. [https://doi.org/10.1016/0092-8674\(83\)90014-4](https://doi.org/10.1016/0092-8674(83)90014-4)
- GILMOUR D. S., LIS J. T. (1986). RNA polymerase II interacts with the promoter region of the noninduced hsp70 gene in *Drosophila melanogaster* cells. *Molecular and Cellular Biology* 6: 3984-3989. <https://doi.org/10.1128/MCB.6.11.3984>
- GLOZAK M. A., SENGUPTA N., ZHANG X., SETO E. (2005). Acetylation and deacetylation of non-histone proteins. *Gene* 363: 15-23. <https://doi.org/10.1016/j.gene.2005.09.010>
- GUO Y. E., MANTEIGA J. C., HENNINGER J. E., SABARI B. R., DALL'AGNESE A., HANNETT N. M., SPILLE J. H., AFEYAN L. K., ZAMUDIO A. V., SHRINIVAS K., ABRAHAM B. J., BOIJA A., et al. (2019). Pol II phosphorylation regulates a switch between transcriptional and splicing condensates. *Nature* 572: 543-548. <https://doi.org/10.1038/s41586-019-1464-0>
- HABERLE V., ARNOLD C. D., PAGANI M., RATH M., SCHERNHUBER K., STARK A. (2019). Transcriptional cofactors display specificity for distinct types of core promoters. *Nature* 570: 122-126. <https://doi.org/10.1038/s41586-019-1210-7>
- HABERLE V., STARK A. (2018). Eukaryotic core promoters and the functional basis of transcription initiation. *Nature Reviews Molecular Cell Biology* 19: 621-637. <https://doi.org/10.1038/s41580-018-0028-8>
- HAMARATOGLU F., AFFOLTER M., PYROWOLAKIS G. (2014). Dpp/BMP signaling in flies: From molecules to biology. *Seminars in Cell & Developmental Biology* 32: 128-136. <https://doi.org/10.1016/j.semcdb.2014.04.036>
- HARRISON M. M., EISEN M. B. (2015). Transcriptional Activation of the Zygotic Genome in *Drosophila*. In *The Maternal-to-Zygotic Transition*. Current Topics in Developmental Biology, Vol. 113. Elsevier, p. 85-112. <https://doi.org/10.1016/bs.ctdb.2015.07.028>
- HARRISON M. M., LI X. Y., KAPLAN T., BOTCHAN M. R., EISEN M. B. (2011). Zelda Binding in the Early *Drosophila melanogaster* Embryo Marks Regions Subsequently Activated at the Maternal-to-Zygotic Transition. *PLoS Genetics* 7: e1002266. <https://doi.org/10.1371/journal.pgen.1002266>
- HEINTZMAN N. D., HON G. C., HAWKINS R. D., KHERADPOUR P., STARK A., HARP L. F., YE Z., LEE L. K., STUART R. K., CHING C. W., CHING K. A., ANTOSIEWICZ-BOURGET J. E., et al. (2009). Histone modifications at human enhancers reflect global cell-type-specific gene expression. *Nature* 459: 108-112. <https://doi.org/10.1038/nature07829>
- HEINTZMAN N. D., STUART R. K., HON G., FU Y., CHING C. W., HAWKINS R. D., BARRERA L. O., VAN CALCAR S., QU C., CHING K. A., WANG W., WENG Z., et al. (2007). Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. *Nature Genetics* 39: 311-318. <https://doi.org/10.1038/ng1966>
- HEIST T., FUKAYA T., LEVINE M. (2019). Large distances separate coregulated genes in living *Drosophila* embryos. *Proceedings of the National Academy of Sciences* 116: 15062-15067. <https://doi.org/10.1073/pnas.1908962116>
- HENDY O., SEREBRENI L., BERGAUER K., MUERTER F., HUBER L., NEMČKO F., STARK A. (2022). Developmental and housekeeping transcriptional programs in *Drosophila* require distinct chromatin remodelers. *Molecular Cell* 82: 3598-3612.e7. <https://doi.org/10.1016/j.molcel.2022.08.019>
- HENIKOFF S., SHILATIFARD A. (2011). Histone modification: cause or cog? *Trends in Genetics* 27: 389-396. <https://doi.org/10.1016/j.tig.2011.06.006>
- HENNINGER J. E., OKSUZ O., SHRINIVAS K., SAGI I., LEROY G., ZHENG M. M., ANDREWS J. O., ZAMUDIO A. V., LAZARIS C., HANNETT N. M., LEE T. I., SHARP P. A., et al. (2021). RNA-Mediated Feedback Control of Transcriptional Condensates. *Cell* 184: 207-225.e24. <https://doi.org/10.1016/j.cell.2020.11.030>
- HENRIQUEST., GILCHRIST D. A., NECHAEV S., BERN M., MUSE G. W., BURKHOLDER A., FARGO D. C., ADELMAN K. (2013). Stable Pausing by RNA Polymerase II Provides an Opportunity to Target and Integrate Regulatory Signals. *Molecular Cell* 52: 517-528. <https://doi.org/10.1016/j.molcel.2013.10.001>
- HNISZ D., SHRINIVAS K., YOUNG R. A., CHAKRABORTY A. K., SHARP P. A. (2017). A Phase Separation Model for Transcriptional Control. *Cell* 169: 13-23. <https://doi.org/10.1016/j.cell.2017.02.007>
- HOGG S. J., MOTORNA O., CLUSE L. A., JOHANSON T. M., COUGHLAN H. D., RAVIRAM R., MYERS R. M., COSTACURTA M., TODOROVSKI I., PIJPERS L., BUJLOSEVIC S., WILLIAMS T., et al. (2021). Targeting histone acetylation dynamics and oncogenic transcription by catalytic P300/CBP inhibition. *Molecular Cell* 81: 2183-2200.e13. <https://doi.org/10.1016/j.molcel.2021.04.015>
- HOLMQVIST P. H., BOIJA A., PHILIP P., CRONA F., STENBERG P., MANNERVIK M. (2012). Preferential Genome Targeting of the CBP Co-Activator by Rel and Smad Proteins in Early *Drosophila melanogaster* Embryos. *PLoS Genetics* 8: e1002769. <https://doi.org/10.1371/journal.pgen.1002769>
- HONG J. W., HENDRIX D. A., LEVINE M. S. (2008a). Shadow Enhancers as a Source of Evolutionary Novelty. *Science* 321: 1314-1314. <https://doi.org/10.1126/science.1160631>
- HONG J. W., HENDRIX D. A., PAPATSENKO D., LEVINE M. S. (2008b). How the Dorsal gradient works: Insights from postgenome technologies. *Proceedings of the National Academy of Sciences* 105: 20072-20076. <https://doi.org/10.1073/pnas.0806476105>

- HORNUNG G., BAR-ZIV R., ROSIN D., TOKURIKI N., TAWFIK D. S., OREN M., BARKAI N. (2012). Noise–mean relationship in mutated promoters. *Genome Research* 22: 2409-2417. <https://doi.org/10.1101/gr.139378.112>
- HOU C., LI L., QIN Z. S., CORCES V. G. (2012). Gene Density, Transcription, and Insulators Contribute to the Partition of the Drosophila Genome into Physical Domains. *Molecular Cell* 48: 471-484. <https://doi.org/10.1016/j.molcel.2012.08.031>
- HSIEH T.H. S., CATTOGLIO C., SLOBODYANYUK E., HANSEN A. S., DARZACQ X., TJIAN R. (2022). Enhancer–promoter interactions and transcription are largely maintained upon acute loss of CTCF, cohesin, WAPL or YY1. *Nature Genetics* 54: 1919-1932. <https://doi.org/10.1038/s41588-022-01223-8>
- HSIEH T.H. S., CATTOGLIO C., SLOBODYANYUK E., HANSEN A. S., RANDO O. J., TJIAN R., DARZACQ X. (2020). Resolving the 3D Landscape of Transcription-Linked Mammalian Chromatin Folding. *Molecular Cell* 78: 539-553.e8. <https://doi.org/10.1016/j.molcel.2020.03.002>
- HUANG A., AMOURDA C., ZHANG S., TOLWINSKI N. S., SAUNDERS T. E. (2017). Decoding temporal interpretation of the morphogen Bicoid in the early Drosophila embryo. *eLife* 6: e26258. <https://doi.org/10.7554/eLife.26258>
- HUG C. B., GRIMALDI A. G., KRUSE K., VAQUERIZAS J. M. (2017). Chromatin Architecture Emerges during Zygotic Genome Activation Independent of Transcription. *Cell* 169: 216-228.e19. <https://doi.org/10.1016/j.cell.2017.03.024>
- HUNT G., BOIJA A., MANNERVIK M. (2022). p300/CBP sustains Polycomb silencing by non-enzymatic functions. *Molecular Cell* 82: 3580-3597.e9. <https://doi.org/10.1016/j.molcel.2022.09.005>
- HUNT G., VAIDR., PIROGOV S., PFABA A., ZIEGENHAIN C., SANDBERG R., REIMEGÅRD J., MANNERVIK M. (2024). Tissue-specific RNA Polymerase II promoter-proximal pause release and burst kinetics in a Drosophila embryonic patterning network. *Genome Biology* 25: 2. <https://doi.org/10.1186/s13059-023-03135-0>
- IBORRA F. J., POMBO A., JACKSON D. A., COOK P. R. (1996). Active RNA polymerases are localized within discrete transcription 'factories' in human nuclei. *Journal of Cell Science* 109: 1427-1436. <https://doi.org/10.1242/jcs.109.6.1427>
- ING-SIMMONS E., VAID R., BING X. Y., LEVINE M., MANNERVIK M., VAQUERIZAS J. M. (2021). Independence of chromatin conformation and gene regulation during Drosophila dorsoventral patterning. *Nature Genetics* 53: 487-499. <https://doi.org/10.1038/s41588-021-00799-x>
- IRIZARRY J., STATHOPOULOS A. (2021). Dynamic patterning by morphogens illuminated by cis-regulatory studies. *Development* 148: dev196113. <https://doi.org/10.1242/dev.196113>
- JANG M. K., MOCHIZUKI K., ZHOU M., JEONG H. S., BRADY J. N., OZATO K. (2005). The Bromodomain Protein Brd4 Is a Positive Regulatory Component of P-TEFb and Stimulates RNA Polymerase II-Dependent Transcription. *Molecular Cell* 19: 523-534. <https://doi.org/10.1016/j.molcel.2005.06.027>
- JERKOVIC I., CAVALLI G. (2021). Understanding 3D genome organization by multi-disciplinary methods. *Nature Reviews Molecular Cell Biology* 22: 511-528. <https://doi.org/10.1038/s41580-021-00362-w>
- JIANG N., EMBERLY E., CUVIER O., HART C. M. (2009). Genome-Wide Mapping of Boundary Element-Associated Factor (BEAF) Binding Sites in Drosophila melanogaster Links BEAF to Transcription. *Molecular and Cellular Biology* 29: 3556-3568. <https://doi.org/10.1128/MCB.01748-08>
- JIANG Y., ZHANG N. R., LI M. (2017). SCALE: modeling allele-specific gene expression by single-cell RNA sequencing. *Genome Biology* 18: 74. <https://doi.org/10.1186/s13059-017-1200-8>
- JIN F., LI Y., DIXON J. R., SELVARAJ S., YE Z., LEE A. Y., YEN C.A., SCHMITT A. D., ESPINOZA C. A., REN B. (2013). A high-resolution map of the three-dimensional chromatin interactome in human cells. *Nature* 503: 290-294. <https://doi.org/10.1038/nature12644>
- JONKERS I., KWAK H., LIS J. T. (2014). Genome-wide dynamics of Pol II elongation and its interplay with promoter proximal pausing, chromatin, and exons. *eLife* 3: e02407. <https://doi.org/10.7554/eLife.02407>
- JONKERS I., LIS J. T. (2015). Getting up to speed with transcription elongation by RNA polymerase II. *Nature Reviews Molecular Cell Biology* 16: 167-177. <https://doi.org/10.1038/nrm3953>
- JOO Y. J., FICARRO S. B., SOARES L. M., CHUN Y., MARTO J. A., BURATOWSKI S. (2017). Downstream promoter interactions of TFIID TAFs facilitate transcription reinitiation. *Genes & Development* 31: 2162-2174. <https://doi.org/10.1101/gad.306324.117>
- KANHERE A., VIIRI K., ARAÚJO C. C., RASAIYAAH J., BOUWMAN R. D., WHYTE W. A., PEREIRA C. F., BROOKES E., WALKER K., BELL G. W., POMBO A., FISHER A. G., et al. (2010). Short RNAs Are Transcribed from Repressed Polycomb Target Genes and Interact with Polycomb Repressive Complex-2. *Molecular Cell* 38: 675-688. <https://doi.org/10.1016/j.molcel.2010.03.019>
- KARAIKOS N., WAHLE P., ALLES J., BOLTENGAGEN A., AYOUB S., KIPAR C., KOCKS C., RAJEWSKY N., ZINZEN R. P. (2017). The Drosophila embryo at single-cell transcriptome resolution. *Science* 358: 194-199. <https://doi.org/10.1126/science.aan3235>
- KARR J. P., FERRIE J. J., TJIAN R., DARZACQ X. (2022). The transcription factor activity gradient (TAG) model: contemplating a contact-independent mechanism for enhancer–promoter communication. *Genes & Development* 36: 7-16. <https://doi.org/10.1101/gad.349160.121>
- KAUSHAL A., DORIER J., WANG B., MOHANA G., TASCHNER M., COUSIN P., WARIDEL P., ISELI C., SEMENOVA A., RESTREPO S., GUEX N., AIDEN E. L., et al. (2022). Essential role of Cp190 in physical and regulatory boundary formation. *Science Advances* 8: eabl8834. <https://doi.org/10.1126/sciadv.abl8834>
- KAUSHAL A., MOHANA G., DORIER J., ÖZDEMIRI, OMER A., COUSIN P., SEMENOVA A., TASCHNER M., DERGAI O., MARZETTA F., ISELI C., ELIAZ Y., et al. (2021). CTCF loss has limited effects on global genome architecture in Drosophila despite critical regulatory functions. *Nature Communications* 12: 1011. <https://doi.org/10.1038/s41467-021-21366-2>
- KAWASAKI K., FUKAYA T. (2023). Functional coordination between transcription factor clustering and gene activity. *Molecular Cell* 83: 1605-1622.e9. <https://doi.org/10.1016/j.molcel.2023.04.018>
- KIM J., MARIONI J. C. (2013). Inferring the kinetics of stochastic gene expression from single-cell RNA-sequencing data. *Genome Biology* 14: R7. <https://doi.org/10.1186/gb-2013-14-1-r7>
- KOENECKE N., JOHNSTON J., GAERTNER B., NATARAJAN M., ZEITLINGER J. (2016). Genome-wide identification of Drosophila dorso-ventral enhancers by differential histone acetylation analysis. *Genome Biology* 17: 196. <https://doi.org/10.1186/s13059-016-1057-2>
- KOENECKE N., JOHNSTON J., HE Q., MEIER S., ZEITLINGER J. (2017). Drosophila poised enhancers are generated during tissue patterning with the help of repression. *Genome Research* 27: 64-74. <https://doi.org/10.1101/gr.209486.116>
- KORNBERG R. D., LORCH Y. (2020). Primary Role of the Nucleosome. *Molecular Cell* 79: 371-375. <https://doi.org/10.1016/j.molcel.2020.07.020>
- KOROMILAT, GAO F., IWASAKI Y., HEP, PACHTER L., GERGEN J. P., STATHOPOULOS A. (2020). Odd-paired is a pioneer-like factor that coordinates with Zelda to control gene expression in embryos. *eLife* 9: e59610. <https://doi.org/10.7554/eLife.59610>
- KOSMAN D., IP Y. T., LEVINE M., ARORA K. (1991). Establishment of the Mesoderm-Neuroectoderm Boundary in the Drosophila Embryo. *Science* 254: 118-122. <https://doi.org/10.1126/science.1925551>
- KRAGESTEEN B. K., SPIELMANN M., PALIOU C., HEINRICH V., SCHÖPFLIN R., ESPOSITO A., ANNUNZIATELLA C., BIANCO S., CHIARIELLO A. M., JERKOVIĆ I., HARABULA I., GUCKELBERGER P., et al. (2018). Dynamic 3D chromatin architecture contributes to enhancer specificity and limb morphogenesis. *Nature Genetics* 50: 1463-1473. <https://doi.org/10.1038/s41588-018-0221-x>
- KRIETENSTEIN N., ABRAHAM S., VENEV S. V., ABDENNUR N., GIBCSU J., HSIEH T.H. S., PARSİ K. M., YANG L., MAEHR R., MIRNY L. A., DEKKER J., RANDO O. J. (2020). Ultrastructural Details of Mammalian Chromosome Architecture. *Molecular Cell* 78: 554-565.e7. <https://doi.org/10.1016/j.molcel.2020.03.003>
- KVON E. Z., KAZMAR T., STAMPFEL G., YÁÑEZ-CUNA J. O., PAGANI M., SCHERNHUBER K., DICKSON B. J., STARK A. (2014). Genome-scale functional characterization of Drosophila developmental enhancers in vivo. *Nature* 512: 91-95. <https://doi.org/10.1038/nature13395>
- KWOK R. P. S., LUNDBLAD J. R., CHRIVIA J. C., RICHARDS J. P., BÄCHINGER H. P., BRENNAN R. G., ROBERTS S. G. E., GREEN M. R., GOODMAN R. H. (1994). Nuclear protein CBP is a coactivator for the transcription factor CREB. *Nature* 370: 223-226. <https://doi.org/10.1038/370223a0>
- LAGHA M., BOTHMA J. P., ESPOSITO E., NG S., STEFANIK L., TSUI C., JOHNSTON J., CHEN K., GILMOUR D. S., ZEITLINGER J., LEVINE M. S. (2013). Paused Pol II Coordinates Tissue Morphogenesis in the Drosophila Embryo. *Cell* 153: 976-987. <https://doi.org/10.1016/j.cell.2013.04.045>
- LARSSON A. J. M., JOHNSON P., HAGEMANN-JENSEN M., HARTMANIS L., FARIDANI O. R., REINIUS B., SEGERSTOLPE Å, RIVERA C. M., REN B., SANDBERG R. (2019). Genomic encoding of transcriptional burst kinetics. *Nature* 565: 251-254. <https://doi.org/10.1038/s41586-018-0836-1>



- LEDILY F., BAÙ D., POHL A., VICENT G. P., SERRA F., SORONELLAS D., CASTELLANO G., WRIGHT R. H. G., BALLARE C., FILION G., MARTI-RENO M. A., BEATO M. (2014). Distinct structural transitions of chromatin topological domains correlate with coordinated hormone-induced gene regulation. *Genes & Development* 28: 2151-2162. <https://doi.org/10.1101/gad.241422.114>
- LEATHAM-JENSEN M., UYEHARA C. M., STRAHL B. D., MATERA A. G., DURONIO R. J., MCKAY D. J. (2019). Lysine 27 of replication-independent histone H3.3 is required for Polycomb target gene silencing but not for gene activation. *PLoS Genetics* 15: e1007932. <https://doi.org/10.1371/journal.pgen.1007932>
- LEVINE M. (2010). Transcriptional Enhancers in Animal Development and Evolution. *Current Biology* 20: R754-R763. <https://doi.org/10.1016/j.cub.2010.06.070>
- LEVINE M. (2011). Paused RNA Polymerase II as a Developmental Checkpoint. *Cell* 145: 502-511. <https://doi.org/10.1016/j.cell.2011.04.021>
- LEVOM., RAIMUNDO J., BING X. Y., SISCO Z., BATUT P. J., RYABICHKO S., GREGOR T., LEVINE M. S. (2022). Transcriptional coupling of distant regulatory genes in living embryos. *Nature* 605: 754-760. <https://doi.org/10.1038/s41586-022-04680-7>
- LEYES PORELLO E. A., TRUDEAU R. T., LIM B. (2023). Transcriptional bursting: stochasticity in deterministic development. *Development* 150: dev201546. <https://doi.org/10.1242/dev.201546>
- LI B., CAREY M., WORKMAN J. L. (2007). The Role of Chromatin during Transcription. *Cell* 128: 707-719. <https://doi.org/10.1016/j.cell.2007.01.015>
- LI G., RUAN X., AUERBACH R. K., SANDHU K. S., ZHENG M., WANG P., POH H. M., GOH Y., LIM J., ZHANG J., SIM H. S., PEH S. Q., et al. (2012). Extensive Promoter-Centered Chromatin Interactions Provide a Topological Basis for Transcription Regulation. *Cell* 148: 84-98. <https://doi.org/10.1016/j.cell.2011.12.014>
- LI X., TANG X., BING X., CATALANO C., LI T., DOLSTEN G., WU C., LEVINE M. (2023). GAGA-associated factor fosters loop formation in the *Drosophila* genome. *Molecular Cell* 83: 1519-1526.e4. <https://doi.org/10.1016/j.molcel.2023.03.011>
- LI X. Y., HARRISON M. M., VILLALTA J. E., KAPLAN T., EISEN M. B. (2014). Establishment of regions of genomic activity during the *Drosophila* maternal to zygotic transition. *eLife* 3: e03737. <https://doi.org/10.7554/eLife.03737>
- LI X. Y., THOMAS S., SABO P. J., EISEN M. B., STAMATOYANNOPOULOS J. A., BIGGIN M. D. (2011). The role of chromatin accessibility in directing the widespread, overlapping patterns of *Drosophila* transcription factor binding. *Genome Biology* 12: R34. <https://doi.org/10.1186/gb-2011-12-4-r34>
- LIANG H. L., NIEN C. Y., LIU H. Y., METZSTEIN M. K., KIROV N., RUSHLOW C. (2008). The zinc-finger protein Zelda is a key activator of the early zygotic genome in *Drosophila*. *Nature* 456: 400-403. <https://doi.org/10.1038/nature07388>
- LIBERMAN L. M., REEVES G. T., STATHOPOULOS A. (2009). Quantitative imaging of the Dorsal nuclear gradient reveals limitations to threshold-dependent patterning in *Drosophila*. *Proceedings of the National Academy of Sciences* 106: 22317-22322. <https://doi.org/10.1073/pnas.0906227106>
- LIM B., HEIST T., LEVINE M., FUKAYA T. (2018). Visualization of Transvection in Living *Drosophila* Embryos. *Molecular Cell* 70: 287-296.e6. <https://doi.org/10.1016/j.molcel.2018.02.029>
- LIM B., LEVINE M. S. (2021). Enhancer-promoter communication: hubs or loops?. *Current Opinion in Genetics & Development* 67: 5-9. <https://doi.org/10.1016/j.gde.2020.10.001>
- LIU B., XU Q., WANG Q., FENG S., LAI F., WANG P., ZHENG F., XIANG Y., WU J., NIE J., QIU C., XIA W., et al. (2020). The landscape of RNA Pol II binding reveals a stepwise transition during ZGA. *Nature* 587: 139-144. <https://doi.org/10.1038/s41586-020-2847-y>
- LONG H. K., PRESCOTT S. L., WYSOCKA J. (2016). Ever-Changing Landscapes: Transcriptional Enhancers in Development and Evolution. *Cell* 167: 1170-1187. <https://doi.org/10.1016/j.cell.2016.09.018>
- LÓPEZ-OTÍN C., BLASCO M. A., PARTRIDGE L., SERRANO M., KROEMER G. (2013). The Hallmarks of Aging. *Cell* 153: 1194-1217. <https://doi.org/10.1016/j.cell.2013.05.039>
- LOTT S. E., VILLALTA J. E., SCHROTH G. P., LUO S., TONKIN L. A., EISEN M. B. (2011). Noncanonical Compensation of Zygotic X Transcription in Early *Drosophila* melanogaster Development Revealed through Single-Embryo RNA-Seq. *PLoS Biology* 9: e1000590. <https://doi.org/10.1371/journal.pbio.1000590>
- LU H., YU D., HANSEN A. S., GANGULY S., LIU R., HECKERT A., DARZACQ X., ZHOU Q. (2018). Phase-separation mechanism for C-terminal hyperphosphorylation of RNA polymerase II. *Nature* 558: 318-323. <https://doi.org/10.1038/s41586-018-0174-3>
- LUPIÁÑEZ D. G., KRAFT K., HEINRICH V., KRAWITZ P., BRANCATI F., KLOPOCKI E., HORN D., KAYSERILI H., OPITZ J. M., LAXOVA R., SANTOS-SIMARRO F., GILBERT-DUSSARDIER B., et al. (2015). Disruptions of Topological Chromatin Domains Cause Pathogenic Rewiring of Gene-Enhancer Interactions. *Cell* 161: 1012-1025. <https://doi.org/10.1016/j.cell.2015.04.004>
- MA J., HE F., XIE G., DENG W. M. (2016). Maternal AP determinants in the *Drosophila* oocyte and embryo. *WIREs Developmental Biology* 5: 562-581. <https://doi.org/10.1002/wdev.235>
- MA L., GAO Z., WU J., ZHONG B., XIE Y., HUANG W., LIN Y. (2021). Co-condensation between transcription factor and coactivator p300 modulates transcriptional bursting kinetics. *Molecular Cell* 81: 1682-1697.e7. <https://doi.org/10.1016/j.molcel.2021.01.031>
- MANNERVIK M., NIBU Y., ZHANG H., LEVINE M. (1999). Transcriptional Co-regulators in Development. *Science* 284: 606-609. <https://doi.org/10.1126/science.284.5414.606>
- MARKSTEIN M., MARKSTEIN P., MARKSTEIN V., LEVINE M. S. (2002). Genome-wide analysis of clustered Dorsal binding sites identifies putative target genes in the *Drosophila* embryo. *Proceedings of the National Academy of Sciences* 99: 763-768. <https://doi.org/10.1073/pnas.012591199>
- MATEO L. J., MURPHY S. E., HAFNER A., CINQUINI I. S., WALKER C. A., BOETTIGER A. N. (2019). Visualizing DNA folding and RNA in embryos at single-cell resolution. *Nature* 568: 49-54. <https://doi.org/10.1038/s41586-019-1035-4>
- MCDANIEL S. L., GIBSON T. J., SCHULZ K. N., FERNANDEZ GARCIA M., NEVIL M., JAIN S. U., LEWIS P. W., ZARET K. S., HARRISON M. M. (2019). Continued Activity of the Pioneer Factor Zelda Is Required to Drive Zygotic Genome Activation. *Molecular Cell* 74: 185-195.e4. <https://doi.org/10.1016/j.molcel.2019.01.014>
- MCKAY D. J., KLUSZA S., PENKE T. J. R., MEERS M. P., CURRY K. P., MCDANIEL S. L., MALEK P. Y., COOPER S. W., TATOMER D. C., LIEB J. D., STRAHL B. D., DURONIO R. J., et al. (2015). Interrogating the Function of Metazoan Histones using Engineered Gene Clusters. *Developmental Cell* 32: 373-386. <https://doi.org/10.1016/j.devcel.2014.12.025>
- MCSWIGGEN D. T., MIR M., DARZACQ X., TJIAN R. (2019). Evaluating phase separation in live cells: diagnosis, caveats, and functional consequences. *Genes & Development* 33: 1619-1634. <https://doi.org/10.1101/gad.331520.119>
- MIAO L., TANG Y., BONNEAU A. R., CHAN S. H., KOJIMA M. L., POWNALL M. E., VEJNAR C. E., GAO F., KRISHNASWAMY S., HENDRY C. E., GIRALDEZ A. J. (2022). The landscape of pioneer factor activity reveals the mechanisms of chromatin reprogramming and genome activation. *Molecular Cell* 82: 986-1002.e9. <https://doi.org/10.1016/j.molcel.2022.01.024>
- MILLÁN-ZAMBRANO G., BURTON A., BANNISTER A. J., SCHNEIDER R. (2022). Histone post-translational modifications – cause and consequence of genome function. *Nature Reviews Genetics* 23: 563-580. <https://doi.org/10.1038/s41576-022-00468-7>
- MILLER O. L. Jr., MCKNIGHT S. L. (1979). Post-replicative nonribosomal transcription units in *D. melanogaster* embryos. *Cell* 17: 551-563. [https://doi.org/10.1016/0092-8674\(79\)90263-0](https://doi.org/10.1016/0092-8674(79)90263-0)
- MIR M., STADLER M. R., ORTIZ S. A., HANNON C. E., HARRISON M. M., DARZACQ X., EISEN M. B. (2018). Dynamic multifactor hubs interact transiently with sites of active transcription in *Drosophila* embryos. *eLife* 7: e40497. <https://doi.org/10.7554/eLife.40497>
- MITO Y., HENIKOFF J. G., HENIKOFF S. (2007). Histone Replacement Marks the Boundaries of cis-Regulatory Domains. *Science* 315: 1408-1411. <https://doi.org/10.1126/science.1134004>
- MORGAN M. A. J., SHILATIFARDA. (2020). Reevaluating the roles of histone-modifying enzymes and their associated chromatin modifications in transcriptional regulation. *Nature Genetics* 52: 1271-1281. <https://doi.org/10.1038/s41588-020-00736-4>
- MUSE G. W., GILCHRIST D. A., NECHAEV S., SHAH R., PARKER J. S., GRISSOM S. F., ZEITLINGER J., ADELMAN K. (2007). RNA polymerase is poised for activation across the genome. *Nature Genetics* 39: 1507-1511. <https://doi.org/10.1038/ng.2007.21>
- NARITA T., HIGASHIJIMA Y., KILIC S., LIEBNERT., WALTER J., CHOUDHARY C. (2023). Acetylation of histone H2B marks active enhancers and predicts CBP/p300 target genes. *Nature Genetics* 55: 679-692. <https://doi.org/10.1038/s41588-023-01348-4>
- NARITA T., ITO S., HIGASHIJIMA Y., CHU W. K., NEUMANN K., WALTER J., SATPATHY S., LIEBNER T., HAMILTON W. B., MASKEY E., PRUS G., SHIBATA M., et al. (2021). Enhancers are activated by p300/CBP activity-dependent PIC assembly, RNAPII recruitment, and pause release. *Molecular Cell* 81: 2166-2182.e6. <https://doi.org/10.1016/j.molcel.2021.03.008>

- NECHAEV S., FARGO D. C., DOS SANTOS G., LIU L., GAO Y., ADELMAN K. (2010). Global Analysis of Short RNAs Reveals Widespread Promoter-Proximal Stalling and Arrest of Pol II in *Drosophila*. *Science* 327: 335-338. <https://doi.org/10.1126/science.1181421>
- NÈGRE N., BROWN C. D., MA L., BRISTOW C. A., MILLER S. W., WAGNER U., KHERADPOUR P., EATON M. L., LORIAUX P., SEALFON R., LI Z., ISHII H., *et al.* (2011). A cis-regulatory map of the *Drosophila* genome. *Nature* 471: 527-531. <https://doi.org/10.1038/nature09990>
- NEUERT G., MUNSKY B., TAN R. Z., TEYTELMAN L., KHAMMASH M., VAN OUDE-NAARDEN A. (2013). Systematic Identification of Signal-Activated Stochastic Gene Regulation. *Science* 339: 584-587. <https://doi.org/10.1126/science.1231456>
- NEUMAYR C., HABERLE V., SEREBRENI L., KARNER K., HENDY O., BOIJA A., HENNINGER J. E., LI C. H., STEJSKAL K., LIN G., BERGAUER K., PAGANI M., *et al.* (2022). Differential cofactor dependencies define distinct types of human enhancers. *Nature* 606: 406-413. <https://doi.org/10.1038/s41586-022-04779-x>
- NIBU Y., ZHANG H., LEVINE M. (1998). Interaction of Short-Range Repressors with *Drosophila* CtBP in the Embryo. *Science* 280: 101-104. <https://doi.org/10.1126/science.280.5360.101>
- NICOLAS D., ZOLLER B., SUTER D. M., NAEF F. (2018). Modulation of transcriptional burst frequency by histone acetylation. *Proceedings of the National Academy of Sciences* 115: 7153-7158. <https://doi.org/10.1073/pnas.1722330115>
- NIEN C. Y., LIANG H. L., BUTCHER S., SUN Y., FU S., GOCHAT T., KIROV N., MANAK J. R., RUSHLOW C. (2011). Temporal Coordination of Gene Networks by Zelda in the Early *Drosophila* Embryo. *PLoS Genetics* 7: e1002339. <https://doi.org/10.1371/journal.pgen.1002339>
- NORA E. P., GOLOBORODKO A., VALTON A. L., GIBCUS J. H., UEBERSOHN A., ABDENNUR N., DEKKER J., MIRNY L. A., BRUNEAU B. G. (2017). Targeted Degradation of CTCF Decouples Local Insulation of Chromosome Domains from Genomic Compartmentalization. *Cell* 169: 930-944.e22. <https://doi.org/10.1016/j.cell.2017.05.004>
- NORA E. P., LAJOIE B. R., SCHULZ E. G., GIORGETTI L., OKAMOTO I., SERVANT N., PILOT T., VAN BERKUM N. L., MEISIG J., SEDAT J., GRIBNAU J., BARILLOT E., *et al.* (2012). Spatial partitioning of the regulatory landscape of the X-inactivation centre. *Nature* 485: 381-385. <https://doi.org/10.1038/nature11049>
- OGIYAMA Y., SCHUETTENGROBER B., PAPAPOPOULOS G. L., CHANG J. M., CAVALLI G. (2018). Polycomb-Dependent Chromatin Looping Contributes to Gene Silencing during *Drosophila* Development. *Molecular Cell* 71: 73-88.e5. <https://doi.org/10.1016/j.molcel.2018.05.032>
- ORTEGA E., RENGACHARI S., IBRAHIM Z., HOGHOUGH N., GAUCHER J., HOEHOUSE A. S., KHOCHBIN S., PANNE D. (2018). Transcription factor dimerization activates the p300 acetyltransferase. *Nature* 562: 538-544. <https://doi.org/10.1038/s41586-018-0621-1>
- OSTERWALDER M., BAROZZI I., TISSIÈRES V., FUKUDA-YUZAWA Y., MANNION B. J., AFZAL S. Y., LEE E. A., ZHU Y., PLAJZER-FRICK I., PICKLE C. S., KATO M., GARVIN T. H., *et al.* (2018). Enhancer redundancy provides phenotypic robustness in mammalian development. *Nature* 554: 239-243. <https://doi.org/10.1038/nature25461>
- PANIGRAHI A., O'MALLEY B. W. (2021). Mechanisms of enhancer action: the known and the unknown. *Genome Biology* 22: 108. <https://doi.org/10.1186/s13059-021-02322-1>
- PAPAGIANNIA, FORÉS M., SHAO W., HES., KOENECKE N., ANDREU M. J., SAMPER N., PAROUSH Z., GONZÁLEZ-CRESPO S., ZEITLINGER J., JIMÉNEZ G. (2018). Capicua controls Toll/IL-1 signaling targets independently of RTK regulation. *Proceedings of the National Academy of Sciences* 115: 1807-1812. <https://doi.org/10.1073/pnas.1713930115>
- PAPATSENKO D., LEVINE M. (2005). Quantitative analysis of binding motifs mediating diverse spatial readouts of the Dorsal gradient in the *Drosophila* embryo. *Proceedings of the National Academy of Sciences* 102: 4966-4971. <https://doi.org/10.1073/pnas.0409414102>
- PECCOUD J., YCART B. (1995). Markovian Modeling of Gene-Product Synthesis. *Theoretical Population Biology* 48: 222-234. <https://doi.org/10.1006/tpbi.1995.1027>
- PENGELLY A. R., COPUR Ö., JÄCKLE H., HERZIG A., MÜLLER J. (2013). A Histone Mutant Reproduces the Phenotype Caused by Loss of Histone-Modifying Factor Polycomb. *Science* 339: 698-699. <https://doi.org/10.1126/science.1231382>
- PENNACCHIO L. A., AHITUV N., MOSES A. M., PRABHAKAR S., NOBREGA M. A., SHOUKRY M., MINOVITSKY S., DUBCHAK I., HOLT A., LEWIS K. D., PLAJZER-FRICK I., AKIYAMA J., *et al.* (2006). In vivo enhancer analysis of human conserved non-coding sequences. *Nature* 444: 499-502. <https://doi.org/10.1038/nature05295>
- PERRY M. W., BOETTIGER A. N., BOTHMA J. P., LEVINE M. (2010). Shadow Enhancers Foster Robustness of *Drosophila* Gastrulation. *Current Biology* 20: 1562-1567. <https://doi.org/10.1016/j.cub.2010.07.043>
- PHILIP P., BOIJA A., VAID R., CHURCHER A. M., MEYERS D. J., COLE P. A., MANNERNVIK M., STENBERG P. (2015). CBP binding outside of promoters and enhancers in *Drosophila melanogaster*. *Epigenetics & Chromatin* 8: 48. <https://doi.org/10.1186/s13072-015-0042-4>
- PIMMETT V. L., DEJEAN M., FERNANDEZ C., TRULLO A., BERTRANDE., RADULESCU O., LAGHA M. (2021). Quantitative imaging of transcription in living *Drosophila* embryos reveals the impact of core promoter motifs on promoter state dynamics. *Nature Communications* 12: 4504. <https://doi.org/10.1038/s41467-021-24461-6>
- PIQUE-REGI R., DEGNER J. F., PAI A. A., GAFFNEY D. J., GILAD Y., PRITCHARD J. K. (2011). Accurate inference of transcription factor binding from DNA sequence and chromatin accessibility data. *Genome Research* 21: 447-455. <https://doi.org/10.1101/gr.112623.110>
- POWNALL M. E., MIAO L., VEJNAR C. E., M'SAAD O., SHERRARD A., FREDERICK M. A., BENITEZ M. D. J., BOSWELL C. W., ZARET K. S., BEWERSDORF J., GIRALDEZ A. J. (2023). Chromatin expansion microscopy reveals nanoscale organization of transcription and chromatin. *Science* 381: 92-100. <https://doi.org/10.1126/science.ade5308>
- PRICE D. H. (2000). P-TEFb, a Cyclin-Dependent Kinase Controlling Elongation by RNA Polymerase II. *Molecular and Cellular Biology* 20: 2629-2634. <https://doi.org/10.1128/MCB.20.8.2629-2634.2000>
- QI D., BERGMAN M., AIHARA H., NIBU Y., MANNERNVIK M. (2008). *Drosophila* Ebi mediates Snail-dependent transcriptional repression through HDAC3-induced histone deacetylation. *The EMBO Journal* 27: 898-909. <https://doi.org/10.1038/emboj.2008.26>
- RADA-IGLESIAS A., BAJPAIR., SWIGUTT., BRUGMANN S. A., FLYNN R. A., WYSOCKA J. (2011). A unique chromatin signature uncovers early developmental enhancers in humans. *Nature* 470: 279-283. <https://doi.org/10.1038/nature09692>
- RAJ A., PESKIN C. S., TRANCHINA D., VARGAS D. Y., TYAGI S. (2006). Stochastic mRNA Synthesis in Mammalian Cells. *PLoS Biology* 4: e309. <https://doi.org/10.1371/journal.pbio.0040309>
- RAMALINGAM V., NATARAJAN M., JOHNSTON J., ZEITLINGER J. (2021). TATA and paused promoters active in differentiated tissues have distinct expression characteristics. *Molecular Systems Biology* 17: e9866. <https://doi.org/10.15252/msb.20209866>
- RAO S. S. P., HUNTLEY M. H., DURAND N. C., STAMENOVA E. K., BOCHKOV I. D., ROBINSON J. T., SANBORN A. L., MACHOLI., OMER A. D., LANDER E. S., AIDEN E. L. (2014). A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping. *Cell* 159: 1665-1680. <https://doi.org/10.1016/j.cell.2014.11.021>
- RAO S. S. P., HUANG S. C., GLENN ST HILAIRE B., ENGREITZ J. M., PEREZ E. M., KIEFFER-KWON K. R., SANBORN A. L., JOHNSTONES E., BASCOM G. D., BOCHKOV I. D., HUANG X., SHAMIM M. S., *et al.* (2017). Cohesin Loss Eliminates All Loop Domains. *Cell* 171: 305-320.e24. <https://doi.org/10.1016/j.cell.2017.09.026>
- REDDINGTON J. P., GARFIELD D. A., SIGALOVA O. M., KARABACAK CALVIELLO A., MARCO-FERRERES R., GIRARDOT C., VIALES R. R., DEGNER J. F., OHLER U., FURLONG E. E. M. (2020). Lineage-Resolved Enhancer and Promoter Usage during a Time Course of Embryogenesis. *Developmental Cell* 55: 648-664.e9. <https://doi.org/10.1016/j.devcel.2020.10.009>
- REEVES G. T., STATHOPOULOS A. (2009). Graded Dorsal and Differential Gene Regulation in the *Drosophila* Embryo. *Cold Spring Harbor Perspectives in Biology* 1: a000836-a000836. <https://doi.org/10.1101/cshperspect.a000836>
- REEVES G. T., TRISNADIN., TRUONG T. V., NAHMAD M., KATZ S., STATHOPOULOS A. (2012). Dorsal-Ventral Gene Expression in the *Drosophila* Embryo Reflects the Dynamics and Precision of the Dorsal Nuclear Gradient. *Developmental Cell* 22: 544-557. <https://doi.org/10.1016/j.devcel.2011.12.007>
- REITER F., WIENERROITHER S., STARK A. (2017). Combinatorial function of transcription factors and cofactors. *Current Opinion in Genetics & Development* 43: 73-81. <https://doi.org/10.1016/j.gde.2016.12.007>
- RICKELSR., HERZ H. M., SZE C. C., CAO K., MORGAN M. A., COLLINGS C. K., GAUSE M., TAKAHASHI Y., WANG L., RENDLEMAN E. J., MARSHALL S. A., KRUEGER A., *et al.* (2017). Histone H3K4 monomethylation catalyzed by Trr and mammalian COMPASS-like proteins at enhancers is dispensable for development and viability. *Nature Genetics* 49: 1647-1653. <https://doi.org/10.1038/ng.3965>

- RICKELS R., SHILATIFARD A. (2018). Enhancer Logic and Mechanics in Development and Disease. *Trends in Cell Biology* 28: 608-630. <https://doi.org/10.1016/j.tcb.2018.04.003>
- RINCON-ARANO H., HALOW J., DELROW J. J., PARKHURST S. M., GROUDINE M. (2012). UpSET Recruits HDAC Complexes and Restricts Chromatin Accessibility and Acetylation at Promoter Regions. *Cell* 151: 1214-1228. <https://doi.org/10.1016/j.cell.2012.11.009>
- RODRIGUEZ J., LARSON D. R. (2020). Transcription in Living Cells: Molecular Mechanisms of Bursting. *Annual Review of Biochemistry* 89: 189-212. <https://doi.org/10.1146/annurev-biochem-011520-105250>
- ROTH S., STEIN D., NÜSSLEIN-VOLHARD C. (1989). A gradient of nuclear localization of the dorsal protein determines dorsoventral pattern in the *Drosophila* embryo. *Cell* 59: 1189-1202. [https://doi.org/10.1016/0092-8674\(89\)90774-5](https://doi.org/10.1016/0092-8674(89)90774-5)
- ROUVIE A. E., LIS J. T. (1988). The RNA polymerase II molecule at the 5' end of the uninduced hsp70 gene of *D. melanogaster* is transcriptionally engaged. *Cell* 54: 795-804. [https://doi.org/10.1016/S0092-8674\(88\)91087-2](https://doi.org/10.1016/S0092-8674(88)91087-2)
- RUSCH J., LEVINE M. (1996). Threshold responses to the dorsal regulatory gradient and the subdivision of primary tissue territories in the *Drosophila* embryo. *Current Opinion in Genetics & Development* 6: 416-423. [https://doi.org/10.1016/S0959-437X\(96\)80062-1](https://doi.org/10.1016/S0959-437X(96)80062-1)
- RUSHLOW C. A., HAN K., MANLEY J. L., LEVINE M. (1989). The graded distribution of the dorsal morphogen is initiated by selective nuclear transport in *Drosophila*. *Cell* 59: 1165-1177. [https://doi.org/10.1016/0092-8674\(89\)90772-1](https://doi.org/10.1016/0092-8674(89)90772-1)
- SABARI B. R., DALL'AGNESE A., BOIJA A., KLEIN I. A., COFFEY E. L., SHRINIVAS K., ABRAHAM B. J., HANNETT N. M., ZAMUDIO A. V., MANTEIGA J. C., LI C. H., GUO Y. E., et al. (2018). Coactivator condensation at super-enhancers links phase separation and gene control. *Science* 361: eaar3958. <https://doi.org/10.1126/science.aar3958>
- SANKAR A., MOHAMMAD F., SUNDARAMURTHY A. K., WANG H., LERDRUP M., TATAR T., HELIN K. (2022). Histone editing elucidates the functional roles of H3K27 methylation and acetylation in mammals. *Nature Genetics* 54: 754-760. <https://doi.org/10.1038/s41588-022-01091-2>
- SANYAL A., LAJOIE B. R., JAIN G., DEKKER J. (2012). The long-range interaction landscape of gene promoters. *Nature* 489: 109-113. <https://doi.org/10.1038/nature11279>
- SATO Y., HILBERT L., ODA H., WAN Y., HEDDLESTON J. M., CHEW T.L., ZABUR-DAEV V., KELLER P., LIONNET T., VASTENHOUW N., KIMURA H. (2019). Histone H3K27 acetylation precedes active transcription during zebrafish zygotic genome activation as revealed by live-cell analysis. *Development* 146: dev179127. <https://doi.org/10.1242/dev.179127>
- SAUNDERS A., CORE L. J., SUTCLIFFE C., LIS J. T., ASHE H. L. (2013). Extensive polymerase pausing during *Drosophila* axis patterning enables high-level and pliable transcription. *Genes & Development* 27: 1146-1158. <https://doi.org/10.1101/gad.215459.113>
- SCHOENFELDER S., FRASER P. (2019). Long-range enhancer-promoter contacts in gene expression control. *Nature Reviews Genetics* 20: 437-455. <https://doi.org/10.1038/s41576-019-0128-0>
- SCHRÖDERS., HERKERE., ITZEN F., HED., THOMAS S., GILCHRIST D. A., KAEHLCKE K., CHO S., POLLARD K. S., CAPRA J. A., SCHNÖLZER M., COLE P. A., et al. (2013). Acetylation of RNA Polymerase II Regulates Growth-Factor-Induced Gene Transcription in Mammalian Cells. *Molecular Cell* 52: 314-324. <https://doi.org/10.1016/j.molcel.2013.10.009>
- SCHUETTENGROBER B., BOURBON H. M., DICROCEL., CAVALLI G. (2017). Genome Regulation by Polycomb and Trithorax: 70 Years and Counting. *Cell* 171: 34-57. <https://doi.org/10.1016/j.cell.2017.08.002>
- SCHUETTENGROBER B., GANAPATHI M., LEBLANC B., PORTOSO M., JASCHEK R., TOLHUIS B., VAN LOHUIZEN M., TANAY A., CAVALLI G. (2009). Functional Anatomy of Polycomb and Trithorax Chromatin Landscapes in *Drosophila* Embryos. *PLoS Biology* 7: e1000013. <https://doi.org/10.1371/journal.pbio.1000013>
- SCHULZ K. N., HARRISON M. M. (2019). Mechanisms regulating zygotic genome activation. *Nature Reviews Genetics* 20: 221-234. <https://doi.org/10.1038/s41576-018-0087-x>
- SCHWARTZ Y. B., KAHN T. G., NIX D. A., LI X. Y., BOURGON R., BIGGIN M., PIRROTTA V. (2006). Genome-wide analysis of Polycomb targets in *Drosophila melanogaster*. *Nature Genetics* 38: 700-705. <https://doi.org/10.1038/ng1817>
- SCHWARZER W., ABDENNUR N., GOLBORODKO A., PEKOWSKA A., FUDENBERG G., LOE-MIE Y., FONSECA N. A., HUBER W., HAERING C. H., MIRNY L., SPITZ F. (2017). Two independent modes of chromatin organization revealed by cohesin removal. *Nature* 551: 51-56. <https://doi.org/10.1038/nature24281>
- SEXTON T., YAFFE E., KENIGSBERG E., BANTIGNIES F., LEBLANC B., HOICHMAN M., PARRINELLO H., TANAY A., CAVALLI G. (2012). Three-Dimensional Folding and Functional Organization Principles of the *Drosophila* Genome. *Cell* 148: 458-472. <https://doi.org/10.1016/j.cell.2012.01.010>
- SHAO W., ZEITLINGER J. (2017). Paused RNA polymerase II inhibits new transcriptional initiation. *Nature Genetics* 49: 1045-1051. <https://doi.org/10.1038/ng.3867>
- SHLYUEVA D., STAMPFEL G., STARK A. (2014). Transcriptional enhancers: from properties to genome-wide predictions. *Nature Reviews Genetics* 15: 272-286. <https://doi.org/10.1038/nrg3682>
- SHRINIVAS K., SABARI B. R., COFFEY E. L., KLEIN I. A., BOIJA A., ZAMUDIO A. V., SCHUIJERS J., HANNETT N. M., SHARP P. A., YOUNG R. A., CHAKRABORTY A. K. (2019). Enhancer Features that Drive Formation of Transcriptional Condensates. *Molecular Cell* 75: 549-561.e7. <https://doi.org/10.1016/j.molcel.2019.07.009>
- SINGH A. P., WU P., RYABICHKO S., RAIMUNDO J., SWAN M., WIESCHAUS E., GREGOR T., TOETTCHER J. E. (2022). Optogenetic control of the Bicoid morphogen reveals fast and slow modes of gap gene regulation. *Cell Reports* 38: 110543. <https://doi.org/10.1016/j.celrep.2022.110543>
- SINHAK K. K., BILOKAPIC S., DUY., MALIK D., HALIC M. (2023). Histone modifications regulate pioneer transcription factor cooperativity. *Nature* 619: 378-384. <https://doi.org/10.1038/s41586-023-06112-6>
- SMALL S., ARNOSTI D. N. (2020). Transcriptional Enhancers in *Drosophila*. *Genetics* 216: 1-26. <https://doi.org/10.1534/genetics.120.301370>
- SMALL S., BLAIR A., LEVINE M. (1992). Regulation of even-skipped stripe 2 in the *Drosophila* embryo. *The EMBO Journal* 11: 4047-4057. <https://doi.org/10.1002/j.1460-2075.1992.tb05498.x>
- SMITH G. D., CHING W. H., CORNEJO-PÁRAMO P., WONG E. S. (2023). Decoding enhancer complexity with machine learning and high-throughput discovery. *Genome Biology* 24: 116. <https://doi.org/10.1186/s13059-023-02955-4>
- SOLURI I. V., ZUMERLING L. M., PAYAN PARRA O. A., CLARK E. G., BLYTHE S. A. (2020). Zygotic pioneer factor activity of Odd-paired/Zic is necessary for late function of the *Drosophila* segmentation network. *eLife* 9: e53916. <https://doi.org/10.7554/eLife.53916>
- SPANGE S., WAGNER T., HEINZEL T., KRÄMER O. H. (2009). Acetylation of non-histone proteins modulates cellular signalling at multiple levels. *The International Journal of Biochemistry & Cell Biology* 41: 185-198. <https://doi.org/10.1016/j.biocel.2008.08.027>
- SPITZ F., FURLONG E. E. M. (2012). Transcription factors: from enhancer binding to developmental control. *Nature Reviews Genetics* 13: 613-626. <https://doi.org/10.1038/nrg3207>
- STADLER M. R., HAINES J. E., EISEN M. B. (2017). Convergence of topological domain boundaries, insulators, and polytene interbands revealed by high-resolution mapping of chromatin contacts in the early *Drosophila melanogaster* embryo. *eLife* 6: e29550. <https://doi.org/10.7554/eLife.29550>
- STANOJEVIC D., SMALL S., LEVINE M. (1991). Regulation of a Segmentation Stripe by Overlapping Activators and Repressors in the *Drosophila* Embryo. *Science* 254: 1385-1387. <https://doi.org/10.1126/science.1683715>
- STASEVICH T. J., HAYASHI-TAKANAKA Y., SATO Y., MAEHARA K., OHKAWA Y., SAKATA-SOGAWA K., TOKUNAGA M., NAGASE T., NOZAKI N., MCNALLY J. G., KIMURA H. (2014). Regulation of RNA polymerase II activation by histone acetylation in single living cells. *Nature* 516: 272-275. <https://doi.org/10.1038/nature13714>
- STATHOPOULOS A., LEVINE M. (2002). Dorsal Gradient Networks in the *Drosophila* Embryo. *Developmental Biology* 246: 57-67. <https://doi.org/10.1006/dbio.2002.0652>
- STATHOPOULOS A., LEVINE M. (2004). Whole-genome analysis of *Drosophila* gastrulation. *Current Opinion in Genetics & Development* 14: 477-484. <https://doi.org/10.1016/j.gde.2004.07.004>
- STATHOPOULOS A., VAN DRENT M., ERIVES A., MARKSTEIN M., LEVINE M. (2002). Whole-Genome Analysis of Dorsal-Ventral Patterning in the *Drosophila* Embryo. *Cell* 111: 687-701. [https://doi.org/10.1016/S0092-8674\(02\)01087-5](https://doi.org/10.1016/S0092-8674(02)01087-5)
- STEIN C. B., FIELD A. R., MIMOSO C. A., ZHAO C. C., HUANG K. L., WAGNER E. J., ADELMAN K. (2022). Integrator endonuclease drives promoter-proximal termination at all RNA polymerase II-transcribed loci. *Molecular Cell* 82: 4232-4245.e11. <https://doi.org/10.1016/j.molcel.2022.10.004>
- STEIN D. S., STEVENS L. M. (2014). Maternal control of the *Drosophila* dorsal-ventral body axis. *WIREs Developmental Biology* 3: 301-330. <https://doi.org/10.1002/wdev.138>

- STEWART R. (1987). Dorsal, an Embryonic Polarity Gene in *Drosophila*, Is Homologous to the Vertebrate Proto-Oncogene, *c-rel*. *Science* 238: 692-694. <https://doi.org/10.1126/science.3118464>
- STEWART R. (1989). Relocalization of the dorsal protein from the cytoplasm to the nucleus correlates with its function. *Cell* 59: 1179-1188. [https://doi.org/10.1016/0092-8674\(89\)90773-3](https://doi.org/10.1016/0092-8674(89)90773-3)
- STOCK J. K., GIADROSSI S., CASANOVA M., BROOKES E., VIDAL M., KOSEKI H., BROCKDORFF N., FISHER A. G., POMBO A. (2007). Ring1-mediated ubiquitination of H2A restrains poised RNA polymerase II at bivalent genes in mouse ES cells. *Nature Cell Biology* 9: 1428-1435. <https://doi.org/10.1038/ncb1663>
- SUN Y., NIEN C. Y., CHEN K., LIU H. Y., JOHNSTON J., ZEITLINGER J., RUSHLOW C. (2015). Zelda overcomes the high intrinsic nucleosome barrier at enhancers during *Drosophila* zygotic genome activation. *Genome Research* 25: 1703-1714. <https://doi.org/10.1101/gr.192542.115>
- SUTER D. M., MOLINA N., GATFIELD D., SCHNEIDER K., SCHIBLER U., NAEF F. (2011). Mammalian Genes Are Transcribed with Widely Different Bursting Kinetics. *Science* 332: 472-474. <https://doi.org/10.1126/science.1198817>
- TANTALE K., GARCIA-OLIVER E., ROBERT M. C., LHOSTIS A., YANG Y., TSANOV N., TOPNO R., GOSTAN T., KOZULIC-PIRHER A., BASU-SHRIVASTAVA M., MUKHERJEE K., SLANINOVA V., et al. (2021). Stochastic pausing at latent HIV-1 promoters generates transcriptional bursting. *Nature Communications* 12: 4503. <https://doi.org/10.1038/s41467-021-24462-5>
- THE modENCODE CONSORTIUM, ROY S., ERNST J., KHARCHENKO P. V., KHERADPOUR P., NEGRE N., EATON M. L., LANDOLIN J. M., BRISTOW C. A., MA L., LIN M. F., WASHIETL S., et al. (2010). Identification of Functional Elements and Regulatory Circuits by *Drosophila* modENCODE. *Science* 330: 1787-1797. <https://doi.org/10.1126/science.1198374>
- THOMPSON P. R., WANG D., WANG L., FULCO M., PEDICONI N., ZHANG D., AN W., GE Q., ROEDER R. G., WONG J., LEVRERO M., SARTORELLI V., et al. (2004). Regulation of the p300 HAT domain via a novel activation loop. *Nature Structural & Molecular Biology* 11: 308-315. <https://doi.org/10.1038/nsmb740>
- THURMAN R. E., RYNES E., HUMBERT R., VIERSTRA J., MAURANO M. T., HAUGEN E., SHEFFIELD N. C., STERGACHIS A. B., WANG H., VERNOT B., GARG K., JOHN S., et al. (2012). The accessible chromatin landscape of the human genome. *Nature* 489: 75-82. <https://doi.org/10.1038/nature11232>
- TIEF F., BANERJEE R., FUC, STRATTON C. A., FANG M., HARTEP. J. (2016). Polycomb inhibits histone acetylation by CBP by binding directly to its catalytic domain. *Proceedings of the National Academy of Sciences* 113: E744-E753. <https://doi.org/10.1073/pnas.1515465113>
- TIE F., BANERJEE R., STRATTON C. A., PRASAD-SINHA J., STEPANIK V., ZLOBIN A., DIAZ M. O., SCACHERI P. C., HARTE P. J. (2009). CBP-mediated acetylation of histone H3 lysine 27 antagonizes *Drosophila* Polycomb silencing. *Development* 136: 3131-3141. <https://doi.org/10.1242/dev.037127>
- TROJANOWSKI J., FRANK L., RADEMACHER A., MÜCKE N., GRIGAITIS P., RIPPE K. (2022). Transcription activation is enhanced by multivalent interactions independent of phase separation. *Molecular Cell* 82: 1878-1893.e10. <https://doi.org/10.1016/j.molcel.2022.04.017>
- TUNNAcliffe E., CHUBB J. R. (2020). What Is a Transcriptional Burst?. *Trends in Genetics* 36: 288-297. <https://doi.org/10.1016/j.tig.2020.01.003>
- ULIANOV S. V., KHRAMEEVA E. E., GAVRILOV A. A., FLYAMER I. M., KOS P., MIKHALEVA E. A., PENIN A. A., LOGACHEVA M. D., IMAKAEV M. V., CHERTOVICH A., GELFAND M. S., SHEVELYOV Y. Y., et al. (2016). Active chromatin and transcription play a key role in chromosome partitioning into topologically associating domains. *Genome Research* 26: 70-84. <https://doi.org/10.1101/gr.196006.115>
- VAID R., WEN J., MANNERVIK M. (2020). Release of promoter-proximal paused Pol II in response to histone deacetylase inhibition. *Nucleic Acids Research* 48: 4877-4890. <https://doi.org/10.1093/nar/gkaa234>
- VANNAM R., SAYILGAN J., OJEDA S., KARAKYRIAKOU B., HU E., KREUZER J., MORRIS R., HERRERA LOPEZ X. I., RAI S., HAAS W., LAWRENCE M., OTT C. J. (2021). Targeted degradation of the enhancer lysine acetyltransferases CBP and p300. *Cell Chemical Biology* 28: 503-514.e12. <https://doi.org/10.1016/j.chembiol.2020.12.004>
- VERVOORT S. J., WELSH S. A., DEVLIN J. R., BARBIERI E., KNIGHT D. A., OFFLEY S., BJELOSEVIC S., COSTACURTA M., TODOROVSKI I., KEARNEY C. J., SANDOW J. J., FAN Z., et al. (2021). The PP2A-Integrator-CDK9 axis fine-tunes transcription and can be targeted therapeutically in cancer. *Cell* 184: 3143-3162.e32. <https://doi.org/10.1016/j.cell.2021.04.022>
- VISEL A., BLOW M. J., LI Z., ZHANG T., AKIYAMA J. A., HOLT A., PLAJZER-FRICK I., SHOUKRY M., WRIGHT C., CHEN F., AFZAL V., REN B., et al. (2009). ChIP-seq accurately predicts tissue-specific activity of enhancers. *Nature* 457: 854-858. <https://doi.org/10.1038/nature07730>
- WAGNER E. J., TONG L., ADELMAN K. (2023). Integrator is a global promoter-proximal termination complex. *Molecular Cell* 83: 416-427. <https://doi.org/10.1016/j.molcel.2022.11.012>
- WALTERS M. C., FIERING S., EIDEMILLER J., MAGIS W., GROUDINE M., MARTIN D. I. (1995). Enhancers increase the probability but not the level of gene expression. *Proceedings of the National Academy of Sciences* 92: 7125-7129. <https://doi.org/10.1073/pnas.92.15.7125>
- WANG H., FAN Z., SHLIAHA P. V., MIELE M., HENDRICKSON R. C., JIANG X., HELIN K. (2023). H3K4me3 regulates RNA polymerase II promoter-proximal pause-release. *Nature* 615: 339-348. <https://doi.org/10.1038/s41586-023-05780-8>
- WANG Z., ZANG C., ROSENFELD J. A., SCHONES D. E., BARSKI A., CUDDAPAH S., CUI K., ROH T. Y., PENG W., ZHANG M. Q., ZHAO K. (2008). Combinatorial patterns of histone acetylations and methylations in the human genome. *Nature Genetics* 40: 897-903. <https://doi.org/10.1038/ng.154>
- WEINERT B. T., NARITA T., SATPATHY S., SRINIVASAN B., HANSEN B. K., SCHÖLZ C., HAMILTON W. B., ZUCCONI B. E., WANG W. W., LIU W. R., BRICKMAN J. M., KESICKI E. A., et al. (2018). Time-Resolved Analysis Reveals Rapid Dynamics and Broad Scope of the CBP/p300 Acetylome. *Cell* 174: 231-244.e12. <https://doi.org/10.1016/j.cell.2018.04.033>
- WONG E. S., ZHENG D., TAN S. Z., BOWER N. I., GARSIDE V., VANWALLEGHEM G., GAITI F., SCOTT E., HOGAN B. M., KIKUCHI K., MCGLINN E., FRANCOIS M., et al. (2020). Deep conservation of the enhancer regulatory code in animals. *Science* 370: eaax8137. <https://doi.org/10.1126/science.aax8137>
- XI H., SHULHA H. P., LIN J. M., VALES T. R., FU Y., BODINE D. M., MCKAY R. D. G., CHENOWETH J. G., TESAR P. J., FUREY T. S., REN B., WENG Z., et al. (2007). Identification and Characterization of Cell Type-Specific and Ubiquitous Chromatin Regulatory Structures in the Human Genome. *PLoS Genetics* 3: e136. <https://doi.org/10.1371/journal.pgen.0030136>
- YAMADA T., YAMAGUCHI Y., INUKAI N., OKAMOTO S., MURA T., HANDA H. (2006). P-TEFb-Mediated Phosphorylation of hSpt5 C-Terminal Repeats Is Critical for Processive Transcription Elongation. *Molecular Cell* 21: 227-237. <https://doi.org/10.1016/j.molcel.2005.11.024>
- YANG Z., YIK J. H. N., CHEN R., HE N., JANG M. K., OZATO K., ZHOU Q. (2005). Recruitment of P-TEFb for Stimulation of Transcriptional Elongation by the Bromodomain Protein Brd4. *Molecular Cell* 19: 535-545. <https://doi.org/10.1016/j.molcel.2005.06.029>
- YOKOSHI M., SEGAWA K., FUKAYA T. (2020). Visualizing the Role of Boundary Elements in Enhancer-Promoter Communication. *Molecular Cell* 78: 224-235.e5. <https://doi.org/10.1016/j.molcel.2020.02.007>
- ZAUGG J. B., SAHLÉN P., ANDERSSON R., ALBERICH-JORDA M., DE LAAT W., DEPLANCKE B., FERRER J., MANDRUP S., NATOLI G., PLEWCZYNSKI D., RADA-IGLESIAS A., SPICUGLIA S. (2022). Current challenges in understanding the role of enhancers in disease. *Nature Structural & Molecular Biology* 29: 1148-1158. <https://doi.org/10.1038/s41594-022-00896-3>
- ZEITLINGER J., STARK A., KELLIS M., HONG J. W., NECHAEV S., ADELMAN K., LEVINE M., YOUNG R. A. (2007a). RNA polymerase stalling at developmental control genes in the *Drosophila melanogaster* embryo. *Nature Genetics* 39: 1512-1516. <https://doi.org/10.1038/ng.2007.26>
- ZEITLINGER J., ZINZEN R. P., STARK A., KELLIS M., ZHANG H., YOUNG R. A., LEVINE M. (2007b). Whole-genome ChIP-chip analysis of Dorsal, Twist, and Snail suggests integration of diverse patterning processes in the *Drosophila* embryo. *Genes & Development* 21: 385-390. <https://doi.org/10.1101/gad.1509607>
- ZHANG T., ZHANG Z., DONG Q., XIONG J., ZHU B. (2020). Histone H3K27 acetylation is dispensable for enhancer activity in mouse embryonic stem cells. *Genome Biology* 21: 45. <https://doi.org/10.1186/s13059-020-01957-w>
- ZHANG Y., BROWN K., YU Y., IBRAHIM Z., ZANDIAN M., XUAN H., INGERSOLL S., LEE T., EBMEIER C. C., LIU J., PANNE D., SHI X., et al. (2021). Nuclear condensates of p300 formed through the structured catalytic core can act as a storage pool of p300 with reduced HAT activity. *Nature Communications* 12: 4618. <https://doi.org/10.1038/s41467-021-24950-8>
- ZOLLER B., LITTLE S. C., GREGOR T. (2018). Diverse Spatial Expression Patterns Emerge from Unified Kinetics of Transcriptional Bursting. *Cell* 175: 835-847.e25. <https://doi.org/10.1016/j.cell.2018.09.056>