Emerging roles of transcriptional enhancers in chromatin looping and promoter-proximal pausing of RNA polymerase II

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Initiation and regulation of transcription by RNA polymerase II (RNAPII) in eukaryotes rely on the transcriptional regulatory elements. Promoters and enhancers share similar architectures and functions, and the prevailing view is that they can initiate bidirectional transcription. We summarize functional roles of enhancer transcription and possible mechanisms in enhancer–promoter communication. We discuss the potential roles of enhancer RNAs (eRNAs) in early elongation and highlight that transcriptional enhancers might modulate the release of paused RNAPII via 3D chromatin looping. Emerging evidence suggests that transcriptional enhancers regulate the promoter–proximal pausing of RNAPII, a key rate-limiting step required for productive elongation.

Mammalian promoters are predominantly bidirectional

Early biochemical studies defined the classical promoter as a DNA region required for the accurate initiation of gene expression (1). In yeast, most promoters contain at least one or several components needed for gene activation: an initiator element, a TATA box, and a downstream core promoter (2). Together, these elements ensure the precise assembly of the basal transcription machinery. Accordingly, the minimal DNA region surrounding the transcription start site (TSS)3 necessary for initiating transcription of a gene is called the core promoter. Before the genomic era, the terms promoter, core promoter, and TSS were used interchangeably, particularly to describe in vitro transcription initiation (3) and the unidirectional transcription of protein-coding genes.

Recent genomic studies have unexpectedly found that more than 60% of the human genome is transcribed, but less than 2% of that transcription originates from coding genes (4, 5). Some of this noncoding transcription can be accounted for by bidirectional transcription taking place near coding genes. It is now recognized that in mammals, the TSSs of genes encoding mRNA frequently generate a pair of oppositely oriented transcripts originating near the same region (6, 7). The unstable, noncoding transcripts originating upstream of the promoter are called promoter upstream transcripts, or PROMPTs (8–13). The paired, bidirectional transcripts are most often generated from genomically accessible regions that display DNase I hypersensitivity. These DNase I hypersensitive sites (DHSs) are typically depleted of nucleosomes, the fundamental subunit of chromatin, resulting in nucleosome-depleted regions (NDRs) or nucleosome-free regions (NFRs). Bidirectional transcripts from mammalian genomes typically originate from either end of an NDR but are not scattered throughout the NDR (7, 9, 13, 14). This led to a new understanding of mammalian promoters as NDRs that support bidirectional transcription initiated from two oppositely oriented core promoters (3, 15). Either side of the core promoter can facilitate the assembly of RNA polymerase II preinitiation complexes (PICs) (Fig. 1, upper panel).

The pervasiveness of bidirectional promoters in mammalian systems raises several crucial questions. Are there distinct biological functions for bidirectional promoters in contrast to unidirectional promoters? What are the specific and common features of bidirectional promoters compared with unidirectional promoters? Do certain classes of bidirectional promoters and their modularity have specific functional properties in terms of DNA sequence consensus and the general transcription factors that bind to them? For example, it will be important to determine the bidirectional modular differences, if any, between active promoter modules of SAGA and TFIID-dependent genes. TATA-binding protein (TBP) preferentially binds “TATA-less” promoters when it is part of the multisubunit TFIID complex (16), whereas the SAGA complex directs TBP to promoters with obvious TATA sequences when TBP is absent from the TFIID complex (17–19). In yeast, “TATA-less” promoters predominate (about 80–90% of all genes), and are more prevalent among ubiquitously expressed “housekeeping”
In contrast, obvious TATA boxes are present at only some promoters that switch between repressed and highly active states, a phenomenon seen in many “developmental genes” (23). Chromatin immunoprecipitation combined with exonuclease digestion (ChIP-exo) was used to map the sites engaged by PICs at high resolution in Saccharomyces cerevisiae (16). This study revealed that two distinct modes of mRNA transcription initiation may exist, depending upon the relationship to the +1 nucleosome near promoters. The first nucleosome in the transcribed region often blocks access to promoters containing obvious TATA boxes. Such promoters may then depend on the SAGA complex to facilitate nucleosome removal for the efficient binding of TBP, RNAPII, and its associated factors. In contrast, in TFIID-dependent promoters, the +1 nucleosome is located downstream of the TSSs. In this case, TBP-associated factors form TFIID with TBP, and interact with DNA sequences downstream of a noncanonical, TATA-like consensus sequence that differs from a TATA box by two or more bases (16). Despite these findings, more recent studies argue against distinct classes of SAGA- and TFIID-dependent genes (24, 25). Warfield et al. (25) found that nearly all yeast-coding genes strongly depend on TFIID at both TATA and TATA-less promoters. SAGA complexes were found at the regulatory regions of both SAGA- and TFIID-dominated genes.
Enhancers as cis-regulatory DNA elements in gene activation

Enhancers are distal sequences that lie upstream or downstream of the core promoter, and can activate or regulate the level of transcriptional initiation by recruiting transcription factors necessary for PIC assembly at the core promoter (Fig. 1) (26–28). In yeast, upstream-activating sequences, also known as enhancer-like sequences, are required for transcription, and are typically positioned much closer to the core promoter (2, 29). Enhancers act independently of their orientation, and their genomic location is believed to be responsible for the accurate surveillance of spatiotemporal transcription patterns during development and/or in different cell types. For example, the first mammalian enhancer was discovered downstream of the immunoglobulin (Ig) heavy-chain gene, which is necessary for the proper expression of Ig, and only exhibits enhancer activity in lymphocyte-derived cell lines and during B lymphocyte differentiation (30, 31).

Enhancers help recruit RNAPII to promoters and can attract various chromatin-modifying enzymes to DNA to establish and/or maintain an active chromatin conformation via PICs (32). Enhancers can also recruit pioneer factors and lineage-specific transcription factors (TFs) as early as the ESC stage (17–22, 33). Promoters, on the other hand, are less likely to be occupied by developmentally important and lineage-specific TFs (34). Notably, enhancers are frequently marked with H3K4me1 and H3K27ac, but not H3K4me3, unless the enhancer is highly transcribed (35–38). Accordingly, putative enhancers are commonly annotated by comparing the previously observed differences in regulation between SAGA- and TFIIID-dependent genes are due to other properties (25). It is currently unclear whether these promoters have distinct features regulating their bidirectional transcription, and whether their associated bidirectional modularity has any biological/pathological significance during development. It also remains elusive how bidirectional promoters of highly paused and productively elongating genes are regulated, and how different types of promoters communicate with other types of regulatory DNA elements like enhancers. The underlying basis driving mammalian promoters to act bidirectionally or unidirectionally in key biological processes is not clear.

Enhancers and promoters share interchangeable properties

As early as 3 decades ago, researchers reported commonalities between promoters and enhancers. For example, when a tandem 72-bp repeat from SV40 polyomavirus was inserted into a plasmid lacking a promoter, this element—the first discovered enhancer—initiated a low level of transcription, indicating that it can recruit RNAPII through a promoter-like activity (23, 32). Later studies found that intragenic enhancers can serve as alternative tissue-specific gene promoters, producing a class of abundant, spliced, and multixenotic poly(A)+ mRNAs (42). Conversely, promoters also display enhancer-like functions. When stimulated by metal ion, an introduced mouse metallothionein I (Mt1) gene promoter acted as an enhancer by increasing the transcription of an upstream rabbit β-globin gene (43). In addition, recent genome-wide analysis using chromatin interaction analysis with paired-end—tag sequencing (ChIA-PET) detected interactions between promoters of different genes that typically resulted in gene co-expression. These findings implied that promoters behaving like enhancers may be common in transcriptional regulation (44). Notably, most promoter—promoter and enhancer—promoter interactions in mammalian genomes are restricted to megabase-sized local chromatin interaction domains, termed topologically associated domains (TADs) (45). However, Stunnenberg and co-workers (46) identified another class of promoter—promoter interactions, extremely long-range interactions, that result in the dynamic restructuring of chromatin as mouse embryonic stem cells (ESCs) shift between two states of pluripotency. These extremely long-range interactions form during the transition from the naive ground-state to the serum primed-like state, and provide yet another example of a promoter displaying enhancer-like function, this time to control the spatiotemporal regulation of Hox and other developmentally important genes.

It is evident that promoters and enhancers share many common features including their local chromatin architecture, their regulatory landscape, and their common mechanisms to control bidirectional transcription (28). However, promoters and their associated coding genes allow for the robust transcription of stable, spliced, and polyadenylated transcripts not seen in transcripts originating from enhancers. The majority of enhancer-templated RNAs (eRNAs) are short, unstable, unspliced, unpolyadenylated, and noncoding RNAs that are expressed at low levels (4, 5). The sequences used to signal for polyadenylation and splicing are absent in the transcribed enhancer regions, but are present in the coding region. Therefore, eRNA instability appears to be due to the lack of polyadenylation and early termination sites, such that eRNAs from both strands are subject to exosome-mediated degradation (10, 47). At bidirectional promoters, poly(A) sites (PASs) are enriched at the 3’ end of PROMPTs that lack 5’ splice sites or U1 small nuclear ribonucleoprotein recognition sites; consequently, these noncoding transcripts are also subject to exosome-dependent degradation. In contrast, coding transcripts contain 5’ splice sites that bind to the U1 splicing complex preventing PAS-mediated early termination (48, 49). It remains unclear whether the PAS-dependent mechanism mediates the degradation of eRNAs.
Functional enhancers transcribe eRNAs as an active signature

Recent genome-wide studies have shown that RNA pol II recruitment to active enhancers initiates widespread transcription in mammalian genomes (50, 51). eRNAs were initially thought to arise from transcriptional noise due to abundant RNAPII activity, generating “nonspecific” transcripts in physically accessible genomic regions (52). This “nonspecific” transcription model suggests that eRNAs may be a by-product of random transcriptional activity at enhancer loci that are recognized and degraded via either nonsense-mediated decay or the exosome (53, 54). However, evidence for transcribed enhancers in recent genome-wide studies argues that enhancer transcription may be a regulated process that is specific to functionally active enhancers, rather than a random process caused by “background” RNAPII activity. For example, poised enhancers that are bound by co-activators and marked with H3K27me3, but not H3K27ac, lack transcriptional activity in mouse ESCs (Fig. 1, lower panel). These poised enhancers have been proposed to bookmark a limited number of regulatory elements in mouse ESCs, and to be activated in a timely and lineage-specific manner during differentiation (37, 55). By contrast, active enhancers marked with H3K4me1 and H3K27ac are highly transcribed, which also positively correlates with high mRNA levels of linked protein-coding genes (36, 37, 55). In addition, primed enhancers flanked by nucleosomes marked with H3K4me1, but not H3K27ac, are associated with intermediate expression genes that are involved in a broad range of biological processes (36, 55). Collectively, enhancer transcription appears to be a regulated process that takes place only at functionally active enhancers (34, 36, 37, 39, 47, 50, 56).

Enhancer transcription and enhancer–promoter communication

Enhancers may use different but not mutually exclusive ways to communicate with their corresponding promoters depending upon the physical distances between these cis-elements. Possible models of communication include a linking model, a tracking/facilitated tracking or scanning model, and a looping model. In mouse ESCs, TADs contain most of the enhancer–promoter interactions, which range from several kilobases to ~1 Mb, with a median length of 880 kb (57). In the linking model, a number of TFs are recruited sequentially following the binding of a first activator protein (such as pioneer TFs) that induces an open chromatin state at a promoter-proximal sequence during differentiation (58) (Fig. 2A). A chain of TFs then progressively extends along the chromatin fiber from the enhancer to the transcribed gene, and recruits the PIC to the core promoter for transcription initiation. This linking model may only apply to gene regulation between the proximal and core promoter, because this cascade of recruitment may not occur across very long distances.

In the tracking model, the enhancer-bound transcription complexes, including active RNAPII, move toward the target promoter in a unidirectional manner (Fig. 2B) (59). Sometimes enhancer-bound proteins do not leave the enhancer, bringing the enhancer to the promoter during facilitated tracking. This results in progressive loop formation until the loop is stabilized at the target promoter. A classical example for this model stems from an ~70-kb region containing five scattered DHS sites within the human β-globin gene locus (60). This region contains cis-regulatory sequences that confer position-independent activation of linked genes. Such regulatory regions were later named locus control regions (LCRs) (61). LCRs control the expression of a linked gene in a tissue-specific, copy-number-dependent manner. The key evidence for a tracking/facilitated tracking occurring at the β-globin LCR is that transcriptional activity extends across the β-globin LCR, its intervening regions, and into the globin genes (62–64). Similarly, other LCRs such as those that control activation of the human growth hormone (hGH) gene and major histocompatibility complex class II genes in their tissue-specific cell types have also shown RNAPII recruitment and transcriptional activity (65, 66). In the tracking model, the transcripts generated from the intervening sequence between enhancers and promoters are proposed to be passive products of active RNAPII ferried toward the target promoters (56, 67). Therefore, the tracking model requires that transcripts from both enhancers and intervening sequences be unidirectional and transiently expressed. However, genome-wide analyses have revealed that eRNA transcription at the majority of enhancers is bidirectional within confined flanking regions, and eRNA expression is often positively correlated with the expression level of the target gene. Therefore, the tracking model may not be a general mechanism in mammalian genomes, but reflective of a limited number of cases, for example, the regulation of different genes that must be expressed simultaneously within the same gene cluster.

The looping model has been proposed to allow for direct contact of promoters and enhancers over long distances. In this model, the enhancer and promoter make contact by looping out the intervening chromatin (Fig. 2, B and C). The resulting chromatin loops are stabilized by protein–protein interactions. A number of large proteins and protein complexes have been proposed to bridge and direct physical contact between enhancers and promoters, to facilitate both chromatin looping and promoter-proximal pausing of RNAPII. These complexes and proteins include chromatin-remodeling complexes, Mediator, CCCTC-binding factor (CTCF), Cohesin, and many lineage-determining transcription factors (68). eRNAs might also physically participate in establishing or stabilizing enhancer–promoter looping by interacting with either the Cohesin or Mediator complexes (69–71). It is not clear how boundary elements or insulators might restrict the action of enhancers on target promoters. Thus, enhancer–promoter communication is likely a complex and highly regulated process, involving mechanisms from a combination of several working models with different spatial, temporal, and physiological contexts.

Enhancer transcription and eRNAs in gene regulation

Although it is generally agreed that the act of enhancer transcription may have an important biological function in gene regulation, the role of the eRNAs themselves remains controversial. The main debate lies in whether enhancer transcripts have an active role in gene regulation or are merely the by-products of RNAPII transcription. Recruitment of RNAPII to chro-
matin can itself lead to the formation of accessible genomic regions by the "piggybacking" of histone-modifying enzymes via the CTD of RNAPII (65, 72). This may enable RNAPII-mediated transcription to induce active chromatin modifications, or to increase the levels of enhancer-specific histone marks while altering the eRNA transcripts. However, this function of RNAPII does not preclude a direct role for eRNA transcripts in transcriptional regulation.

Initial observations of genome-wide features of active enhancers revealed a number of stimulation-regulated eRNAs in many different mammalian cell types, including neurons, macrophages, and embryonic stem cells (70, 71, 73–75). Interestingly, the levels of these eRNAs often correlate with the expression levels of nearby protein-coding genes. It has been proposed that eRNA synthesis is one of the earliest events relative to mRNA transcription in response to a variety of environmental or developmental stimuli (51, 71, 76–78). Importantly, a number of studies indicate that knockdown of eRNAs results in a substantial down-regulation of their enhancer-targeted genes, suggesting that eRNA transcripts might function in transcriptional activation (70, 71, 74). eRNAs may also participate in chromosomal looping by recruiting Cohesin or Mediator to enhancer regions upon stimulation (70, 71, 79). Support for this idea stems from the finding that a number
of long-noncoding RNAs (lncRNAs), including HOTTIP, CCAT1-L, and LUNAR1, activate their corresponding genes by interacting with their own protein partners that participate in chromosomal looping (80–82). It is noteworthy that a substantial number of lncRNAs are eRNAs. A recent study analyzed functional lncRNAs from the ENCODE project, and identified that 28% of annotated lncRNAs (2,695 of 9,505) overlapped with PreSTIGE database-predicted cell-type–specific enhancers (83), suggesting a subset of eRNAs may be subcategorized as lnc-eRNAs with the potential to interact with distinct proteins involved in chromosomal looping (84). However, a primary difference between eRNA and lncRNA is that lncRNA are stable transcripts and eRNA are not. Some studies suggest eRNAs have roles other than mediating enhancer–promoter looping. These studies indicate that there is a substantial reduction of eRNAs and correlated coding gene expression without significant changes in DNA looping in distinct cell models, including transcription from estrogen receptor–binding sites in MCF7 breast cancer cells and a depolarized neuron model (76, 77). These studies raise the possibility that eRNAs may facilitate RNAPII transcription by increasing chromatin accessibility, or by participating in the process of releasing paused RNAPII without changes in enhancer–promoter interactions (76, 77).

**Emerging roles of eRNAs in promoter-proximal pausing of RNAPII**

A number of recent genome-wide studies have shown that pausing of RNAPII in promoter–proximal regions is a common regulatory step in the productive transcription of many important genes responsive to a variety of developmental or environmental stimuli (85, 86). The pause and release of RNAPII in promoter–proximal DNA regions are essential regulatory steps in early elongation (Fig. 2, C and D). A role for eRNAs in this process has been suggested by Schaukowich et al. (77), who reported that eRNA transcript-dependent regulation during early transcriptional elongation provides a mechanism by which eRNAs, in response to early neuronal induction, directly bind to the NELF-E subunit of negative elongation factor (NELF). NELF mediates RNAPII pausing, and thus facilitates the efficient release of NELF from the target promoter (77). In this activity-regulated neuronal model, transient release of NELF, but not enhancer–promoter interactions or RNAPII recruitment, was impaired by eRNA knockdown, suggesting eRNA transcripts may act as “lure” molecules to facilitate NELF release from paused RNAPII during early transcriptional elongation (Fig. 2D).

Transcriptional elongation by RNAPII is a highly regulated process that requires RNAPII pausing for efficient transcription. The role of eRNAs in pausing and early elongation may depend largely on different developmental or environmental contexts. A recent study from Lai et al. (79) reported that Integrator, a multisubunit complex associated with the CTD of RNAPII, has a role in RNAPII pause and release, and is also required for the biogenesis of eRNAs at enhancers and super-enhancers. Super-enhancers are clusters of multiple transcriptional enhancers in large domains, which often show high levels of RNAPII occupancy and are highly transcribed (87–89). Integrator binds to enhancers and super-enhancers in a tissue- and temporal-specific manner. The catalytic subunit of Integrator has a core RNA endonuclease activity that catalyzes the 3′-end processing of eRNAs required for transcriptional termination of eRNAs upon stimulation (79). Depletion of Integrator subunits reduces the signal-dependent induction of eRNA transcripts and abolishes the associated enhancer–promoter chromatin looping. Interestingly, Integrator depletion also results in an accumulation of eRNA primary transcripts (unprocessed, polyadenylated levels) that bind to transcribing RNAPII at enhancers and super-enhancers upon epidermal growth factor induction, suggesting a possible role of eRNAs in early elongation at Integrator-regulated enhancers and super-enhancers. Multiple eRNAs generated within the same super-enhancers may act as single regulatory modules to control cell identity in development and disease. Accordingly, chromatin looping and promoter-proximal pausing of RNAPII provide a key platform and a potential regulatory step for the convergence of various signaling pathways during early elongation. Additional studies will be required to fully define the functional and biological significance of eRNAs, their in vivo inter–relationship with bidirectional promoters and enhancer transcription, the associated large protein complexes as mediators, and their structural and functional roles in early transcriptional steps, including chromatin looping and promoter-proximal pausing of RNAPII.

It is noteworthy that there is no consensus regarding the function of eRNA transcripts in transcriptional activation. Furthermore, the mechanism underlying the strong correlation between eRNA production and enhancer activity remains unclear. Current studies reporting the functional significance of eRNAs have relied on RNA interference (RNAi) to knock down eRNA transcripts in different human cell lines. However, RNAi approaches are not entirely suitable for these studies. The majority of eRNAs are nuclear, and although RNAi works well in the cytoplasm, it is not efficient in the nucleus (33). Instead, using a polyadenylation signal to cause premature termination might provide a more rigorous way to interrogate eRNA function (90, 91). Indeed, when a poly(A) cassette is inserted near the TSS of an eRNA, it triggers premature transcription termination. A recent study used this approach to investigate how truncation of the lncRNA encoded by Lockd influences transcription of the adjacent Cdkn1b gene in an erythroid cell line (91). They found that Lockd truncation, caused by the inserted poly(A) signal, had no effect on Cdkn1b transcription, whereas CRISPR–Cas9–mediated deletion of the Lockd locus significantly reduced Cdkn1b expression, suggesting an enhancer-like cis-acting mechanism. The eRNA generated from the enhancer-like Lockd locus appeared to be a by-product of local transcriptional activity. Likewise, both promoter deletions and poly(A) cassette insertion into the Blustr lncRNA locus (formerly linc1319) substantially influenced the expression of the nearby Sfbnt2 gene. This influence was dependent upon the transcription and splicing of Blustr (90). Yet, not all lncRNAs are likely to have such specific functions. In a separate study, Lander and co-workers (90) systematically analyzed 12 lncRNA loci in mouse ESCs using a genetic approach based on the classic cis–trans test. They found that five of the 12 lncRNAs significantly affected the expression of a neighboring gene in cis (90). Notably, all five lncRNAs appeared to influence nearby
gene expression via general processes associated with lncRNA production, rather than a sequence-specific function of the lncRNA transcripts. Therefore, more rigorous methods such as the insertion of a poly(A) cassette, in addition to the RNAi approaches, are required to further clarify the cis-trans regulatory roles of eRNA in transcriptional activation. Regardless, it is fair to say that a consensus on the functional significance of eRNAs is lacking, and the relationship between eRNA and enhancer transcriptional activity remains unclear.

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References
1. Smale, S. T., and Kadonaga, J. T. (2003) The RNA polymerase II core promoter. Annu. Rev. Biochem. 72, 449–479 CrossRef Medline
2. Struhl, K. (1987) Promoters, activator proteins, and the mechanism of transcriptional initiation in yeast. Cell 49, 295–297 CrossRef Medline
3. Andersson, R., Chen, Y., Core, L., Lis, J. T., Sandelin, A., and Jensen, T. H. (2015) Human gene promoters are intrinsically bidirectional. Mol. Cell 60, 346–347 CrossRef Medline
4. ENCODE Project Consortium. (2012) An integrated encyclopedia of DNA elements in the human genome. Nature 489, 57–74 CrossRef Medline
5. Djebali, S., Davis, C. A., Merkel, A., Dobin, A., Lassmann, T., Mortazavi, A., Tanzer, A., Lagarde, J., Lin, W., Schlesinger, F., Xue, C., Marinov, G. K., Khatun, J., Williams, B. A., Zaleski, C., et al. (2012) Landscape of transcription in human cells. Nature 489, 101–108 CrossRef Medline
6. Andersson, R., Refsing Andersen, P., Valen, E., Core, L. J., Bornholdt, J., Boyd, M., Heick Jensen, T., and Sandelin, A. (2014) Nuclear stability and transcriptional directionality separate functionally distinct RNA species. Nat. Commun. 5, 5336 CrossRef Medline
7. Core, L. J., Martins, A. L., Danko, C. G., Waters, C. T., Siepel, A., and Lis, J. T. (2014) Analysis of nascent RNA identifies a unified architecture of initiation regions at mammalian promoters and enhancers. Nat. Genet. 46, 1311–1320 CrossRef Medline
8. Almada, A. E., Wu, X., Kriz, A. J., Burge, C. B., and Sharp, P. A. (2013) Promoter directionality is controlled by U1 snRNP and polyadenylation signals. Nature 499, 360–363 CrossRef Medline
9. Core, L. J., Waterfall, J. L., and Lis, J. T. (2008) Nascent RNA sequencing reveals widespread pausing and divergent initiation at human promoters. Science 322, 1845–1848 CrossRef Medline
10. Flynn, R. A., Almada, A. E., Zamudio, J. R., and Sharp, P. A. (2011) Antisense RNA polymerase II divergent transcripts are P-TEFb dependent and substrates for the RNA exosome. Proc. Natl. Acad. Sci. U.S.A. 108, 10460–10465 CrossRef Medline
11. Ntini, E., Jarvelin, A. I., Bornholdt, J., Chen, Y., Boyd, M., Jørgensen, M., Andersson, R., Hoof, L., Schein, A., Andersen, P. R., Andersen, P. K., Preker, P., Valen, E., Zhao, X., Pelechano, V., et al. (2013) Polyadenylation site-induced decay of upstream transcripts enforces promoter directionality. Nat. Struct. Mol. Biol. 20, 923–928 CrossRef Medline
12. Preker, P., Nielsen, J., Kammler, S., Lykke-Andersen, S., Christensen, M. S., Mapendano, C. K., Schierup, M. H., and Jensen, T. H. (2008) RNA exosome depletion reveals transcription upstream of active human promoters. Science 322, 1851–1854 CrossRef Medline
13. Seila, A. C., Calabrese, J. M., Levine, S. S., Yeo, G. W., Rahl, P. B., Flynn, R. A., Young, R. A., and Sharp, P. A. (2008) Divergent transcription from active promoters. Science 322, 1849–1851 CrossRef Medline
14. Scrruggs, B. S., Gilchrist, D. A., Nechaev, S., Muse, G. W., Burkholder, A., Fargo, D. C., and Adelman, K. (2015) Bidirectional transcription arises from two distinct hubs of transcription factor binding and active chromatin. Mol. Cell 58, 1101–1112 CrossRef Medline
15. Kaplan, C. D. (2016) Pairs of promoter pairs in a web of transcription. Nat. Genet. 48, 975–976 CrossRef Medline
16. Basehoar, A. D., Zanton, S. J., and Pugh, B. F. (2004) Identification and distinct regulation of yeast TATA box-containing genes. Cell 116, 699–709 CrossRef Medline
17. Mohibullah, N., and Hahn, S. (2008) Site-specific cross-linking of TBP in vivo and in vitro reveals a direct functional interaction with the SAGA subunit Spt3. Genes Dev. 22, 2994–3006 CrossRef Medline
18. Dudley, A. M., Rougeulle, C., and Winston, F. (1999) The Spt components of SAGA facilitate TBP binding to a promoter at a post-activator-binding step in vivo. Genes Dev. 13, 2940–2945 CrossRef Medline
19. Bhaumik, S. R., and Green, M. R. (2002) Differential requirement of SAGA components for recruitment of TATA-box-binding protein to promoters in vivo. Mol. Cell. Biol. 22, 7365–7371 CrossRef Medline
20. Huisingsa, K. L., and Pugh, B. F. (2004) A genome-wide housekeeping role for TFII D and a highly regulated stress-related role for SAGA in Saccharomyces cerevisiae. Mol. Cell 13, 573–585 CrossRef Medline
21. Lee, T. I., Causton, H. C., Holstege, F. C., Shen, W. C., Hannett, N., Jennnings, E. G., Winston, F., Green, M. R., and Young, R. A. (2000) Redundant roles for the TFID and SAGA complexes in global transcription. Nature 405, 701–704 CrossRef Medline
22. Tirosh, I., and Barkai, N. (2008) Two strategies for gene regulation by promoter nucleosomes. Genome Res. 18, 1084–1091 CrossRef Medline
23. Benoist, C., and Chambon, P. (1981) In vivo sequence requirements of the SV40 early promoter region. Nature 290, 304–310 CrossRef Medline
24. Baptista, T., Grünberg, S., Minoungou, N., Koster, M. J. E., Timmers, H. T. M., Hahn, S., Devys, D., and Tora, L. (2018) SAGA is a general cofactor for RNA polymerase II transcription. Mol. Cell 70, 1163–1164 CrossRef Medline
25. Warfield, L., Ramachandran, S., Baptista, T., Devys, D., Tora, L., and Hahn, S. (2017) Transcription of nearly all yeast RNA polymerase II-transcribed genes is dependent on transcription factor TFII D. Mol. Cell 68, 118–129.e5 CrossRef Medline
26. Akbari, O. S., Bae, E., Johnsen, H., Illulutz, A., Wong, D., and Drewell, R. A. (2008) A novel promoter-tethering element regulates enhancer-driven gene expression at the bithorax complex in the Drosophila embryo. Development 135, 123–131 CrossRef Medline
27. Maniatis, T., Goodbourn, S., and Fischer, J. A. (1987) Regulation of inducible and tissue-specific gene expression. Science 236, 1237–1245 CrossRef Medline
28. Kim, T. K., and Shiekhattar, R. (2015) Architectural and functional commonalities between enhancers and promoters. Cell 162, 948–959 CrossRef Medline
29. Guarente, L. (1988) UASs and enhancers: common mechanism of transcriptional activation in yeast and mammals. Cell 52, 303–305 CrossRef Medline
30. Banerji, J., Olson, L., and Schaffner, W. (1983) A lymphocyte-specific cellular enhancer is located downstream of the joining region in immunoglobulin heavy chain genes. Cell 33, 729–740 CrossRef Medline
31. Gillies, S. D., Morrison, S. L., Oi, V. T., and 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 CrossRef Medline
32. Moreau, P., Hen, R., Wasylyk, B., Everett, R., Gauth, M. P., and Chambon, P. (1981) The SV40 T2 base repair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. Nucleic Acids Res. 9, 6047–6068 CrossRef Medline
33. Roux, B. T., Lindsay, M. A., and Heward, J. A. (2017) Knockdown of nuclear-located enhancer RNAs and long ncRNAs using locked nucleic acid GapmeRs. Methods Mol. Biol. 1468, 11–18 CrossRef Medline
34. Hah, N., Danko, C. G., Core, L., Waterfall, J. J., Siepel, A., Lis, J. T., and Kraus, W. L. (2011) A rapid, extensive, and transient transcriptional response to estrogen signaling in breast cancer cells. Cell 145, 622–634 CrossRef Medline
35. 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., Green, R. D., Crawford, G. E., and Ren, B. (2007) Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat. Genet. 39, 311–318 CrossRef Medline
36. Creighton, M. P., Cheng, A. W., Welstead, G. G., Koositra, T., Carey, B. W., Steine, E. J., Hanna, J., Lodato, M. A., Frampton, G. M., Sharp, P. A., Boyer, L. A., Young, R. A., and Jaenisch, R. (2010) Histone H3K27ac separates active from poised enhancers and predicts developmental state. Proc. Natl. Acad. Sci. U.S.A. 107, 21931–21936 CrossRef Medline

37. Rada-Iglesias, A., Bajpai, R., Swigut, T., Brugmann, S. A., Flynn, R. A., and Wysocka, J. (2011) A unique chromatin signature uncovers early developmental enhancers in humans. Nature 470, 279–283 CrossRef Medline

38. Whyte, A. A., Bilodeau, S., Orlando, D. A., Hoke, H. A., Frampton, G. M., Foster, C. T., Cowley, S. M., and Young, R. A. (2012) Enhancer deconvolution by LSD1 during embryonic stem cell differentiation. Nature 482, 221–225 CrossRef Medline

39. Kaikkonen, M. U., Spann, N. J., Heinz, S., Romanoski, C. E., Allison, K. A., Stender, J. D., Chun, H. B., Tough, D. F., Prinjha, R. K., Benner, C., and Glass, C. K. (2013) Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription. Mol. Cell 51, 310–325 CrossRef Medline

40. Shlyueva, D., Stempfel, G., and Stark, A. (2014) Transcriptional enhancers: from properties to genome-wide predictions. Nat. Rev. Genet. 15, 272–286 CrossRef Medline

41. Rivera, C. M., and Ren, B. (2013) Mapping human epigenomes. Nat. Rev. Genet. 15, 36–46 CrossRef Medline

42. Joshi, O., Wang, S. Y., Kuznetsova, T., Atlasi, Y., Peng, T., Fabre, P. J., Creyghton, M. P., Cheng, A. W., Welstead, G. G., Kooistra, T., Carey, J. D., Chun, H. B., Tough, D. F., Prinjha, R. K., Benner, C., and Glass, C. K. (2013) Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription. Mol. Cell 51, 310–325 CrossRef Medline

43. Serfling, E., Lübbe, A., Dorsch-Häsler, K., and Schaffner, W. (1985) Metal-dependent SV40 viruses containing inducible enhancers. EMBO J. 4, 3581–3589 Medline

44. Kowalczyk, M. S., Hughes, J. R., Garrick, D., Lynch, M. D., Sharpe, J. A., Sloane-Stanley, J. A., McGowan, S. J., De Gobbi, M., Hosseinii, M., Vernimmen, D., Brown, J. M., Gray, N. E., Collavin, L., Gibbons, R. J., Flint, J., et al. (2012) Intragenic enhancers act as alternative promoters. Mol. Cell 45, 447–458 CrossRef Medline

45. Berg, M. G., Singh, L. N., Younis, I., Liu, Q., Pinto, A. M., Kaida, D., Zhang, Z., Cho, S., Sherrill-Mix, S., Wan, L., and Dreyfuss, G. (2012) U1 snRNP determines mRNA length and regulates isoform expression. Cell 150, 53–64 CrossRef Medline

46. Joshi, O., Wang, S. Y., Kuznetsova, T., Atlasi, Y., Peng, T., Fabre, P. J., Habibi, E., Shaik, J., Saeed, S., Harmin, D. A., Laptewicz, M., Barbara-Haley, K., Kuersten, S., Markenscoff-Papadamitriou, E., Kuhl, D., Bito, H., Worley, P. F., Arner, E., Chen, Y., Li, K., Schwarzfischer, L., et al. (2014) An atlas of active enhancers across human cell types and tissues. Nature 507, 455–461 CrossRef Medline

47. Bega, M. G., Singh, L. N., Younis, I., Liu, Q., Pinto, A. M., Kaida, D., Zhang, Z., Cho, S., Sherrill-Mix, S., Wan, L., and Dreyfuss, G. (2012) U1 snRNP determines mRNA length and regulates isoform expression. Cell 150, 53–64 CrossRef Medline

48. De Santa, F., Barozzi, I., Mietton, F., Ghisletti, S., Polletti, S., Tusi, B. K., Muller, H., Ragoussis, I., Wei, C. L., and Natoli, G. (2010) A large fraction of extragenic RNA pol II transcription sites overlap enhancers. PLoS Biol. 8, e1000384 CrossRef Medline

49. Struhl, K. (2007) Transcriptional noise and the fidelity of initiation by RNA polymerase II. Nat. Struct. Mol. Biol. 14, 103–105 CrossRef Medline

50. Wyers, F., Rougemaille, M., Badis, G., Rousselle, J. C., Dufour, M. E., Boulay, J., Régnault, B., Devaux, F., Namane, A., Séraphin, B., Libri, D., and Jacquier, A. (2005) Cryptic pol II transcripts are degraded by a nuclear quality control pathway involving a new poly(A) polymerase. Cell 121, 725–737 CrossRef Medline

51. Zentner, G. E., Tesar, P. J., and Scacheri, P. C. (2011) Epigenetic signatures distinguish multiple classes of enhancers with distinct cellular functions. Genome Res. 21, 1273–1283 CrossRef Medline

52. Hatzis, P., and Talianidis, I. (2002) Dynamic enhancer–promoter communication during differentiation-induced gene activation. Mol. Cell 10, 1467–1477 CrossRef Medline

53. Noonan, J. P., and McCallion, A. S. (2010) Genomics of long-range regulatory elements. Annu. Rev. Genomics Hum. Genet. 11, 1–25 CrossRef Medline

54. Struhl, K. (2007) Transcriptional noise and the fidelity of initiation by RNA polymerase II. Nat. Struct. Mol. Biol. 14, 103–105 CrossRef Medline

55. Blackwood, E. M., and Kadowana, T. J. (1998) Going the distance: a current view of enhancer activation. Science 281, 60–63 CrossRef Medline

56. Grosveld, F., van Assendelft, G. B., Greaves, D. R., and Kollas, G. (1987) Position-independent, high-level expression of the human beta-globin gene in transgenic mice. Cell 51, 975–985 CrossRef Medline

57. Orkin, S. H. (1990) Glubin gene regulation and switching: circa 1990. Cell 63, 669–672 CrossRef Medline

58. Li, W., Notani, D., Ma, Q., Sun, T., Sweeney, C. J., Lee, G. S., Chen, X., Oh, S., Kim, H. S., Glass, C. K., and Rosenfeld, M. G. (2013) Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. Nature 498, 497–501 CrossRef Medline

59. Li, W., Notani, D., Ma, Q., Tantan, B., Nunez, E., Chen, A. Y., Merkurjev, D., Zhang, J., Ohgi, K., Song, H. M., Goh, Y., Lim, J., Flait, J., et al. (2012) Intergenic enhancers provide a topological basis for transcription regulation. Cell 148, 84–98 CrossRef Medline

60. Routledge, S. J., and Proudfoot, N. J. (2002) Definition of transcriptional coactivators involves chromatin communication during differentiation-induced gene activation. Mol. Cell 10, 132–137 CrossRef Medline

61. Masternak, K., Peyraud, N., Krawczyk, M., Barras, E., and Reith, W. (2003) Chromatin remodeling and extragenic transcription at the MHC class II locus control region. Nat. Immunol. 4, 132–137 CrossRef Medline

62. Wang, Q., Carroll, J. S., and Brown, M. (2005) Spatial and temporal recruitment of androgen receptor and its coactivators involves chromosomal looping and polymerase tracking. Mol. Cell 19, 631–642 CrossRef Medline

63. Vernimmen, D., and Bickmore, W. A. (2015) The hierarchy of transcriptional activation: from enhancer to promoter. Trends Genet. 31, 696–708 CrossRef Medline

64. Li, F., Orom, U. A., Cesaroni, M., Beringer, M., Taatjes, D. J., Blobel, G. A., and Shiekhattar, R. (2013) Activating RNAs associate with mediator to enhance chromatin architecture and transcription. Nature 494, 497–501 CrossRef Medline

65. Lai, F., Orom, U. A., Cesaroni, M., Beringer, M., Taatjes, D. J., Blobel, G. A., and Shiekhattar, R. (2013) Activating RNAs associate with mediator to enhance chromatin architecture and transcription. Nature 494, 497–501 CrossRef Medline

66. Heintz, C. L., Fei, T., Chen, Y., Li, T., Gao, Y., Wang, X., Sun, T., Sweeney, C. J., Lee, G. S., Chen, X., Balk, S. P., Liu, X. S., Brown, M., and Kantoff, P. W. (2014) Enhancer RNAs participate in androgen receptor-driven looping that selectively enhances gene activation. Proc. Natl. Acad. Sci. U.S.A. 111, 7319–7324 CrossRef Medline

67. Griboval, E., Diederich, K., Pruzinska, S., Calzolari, R., and Fraser, P. (2000) Intergenic transcription and developmental remodeling of chromatin
subdomains in the human β-globin locus. Mol. Cell 5, 377–386 CrossRefMedline

73. Melo, C. A., Drost, I., Wijchers, P. J., van de Werken, H., de Wit, E., Oude Vrielink, J. A., Elkon, R., Melo, S. A., Léveillé, N., Kalluri, R., de Laat, W., and Agami, R. (2013) eRNAs are required for p53-dependent enhancer activity and gene transcription. Mol. Cell 49, 524–535 CrossRefMedline

74. Mousavi, K., Zare, H., Dell’ors o, S., Grontved, L., Gutierrez-Cruz, G., Derfoul, A., Hager, G. L., and Sartorelli, V. (2013) eRNAs promote transcription by establishing chromatin accessibility at defined genomic loci. Mol. Cell 51, 606–617 CrossRefMedline

75. IIIott, N. E., Heward, J. A., Roux, B., Tsitsiou, E., Fenwick, P. S., Lenzi, L., Goodhead, I., Hertz-Fowler, C., Heger, A., Hall, N., Donnelly, L. E., Sims, D., and Lindsay, M. A. (2014) Long noncoding RNAs and enhancer RNAs regulate the lipopolysaccharide-induced inflammatory response in human monocytes. Nat. Commun. 5, 3979 CrossRefMedline

76. Hah, N., Murakami, S., Nagari, A., Danko, C. G., and Kraus, W. L. (2013) Enhancer transcripts mark active estrogen receptor bindingsites. Genome Res. 23, 1210–1223 CrossRefMedline

77. Schaukowitch, K., Joo, J. Y., Liu, X., Watts, J. K., Martinez, C., and Kim, T. K. (2014) Enhancer RNA facilitates NELF release from immediate early genes. Mol. Cell 56, 29–42 CrossRefMedline

78. Arner, E., Daub, C. O., Vitting-Seerup, K., Andersson, R., Lilje, B., Drablas, F., Lennartsson, A., Rönnérblad, M., Hrydziuszko, O., Vitezic, M., Freeman, T. C., Alhendi, A. M., Arner, P., Axton, R., Baillie, J. K., et al. (2015) Transcribed enhancers lead waves of coordinated transcription in transitioning mammalian cells. Science 347, 1010–1014 CrossRefMedline

79. Lai, F., Gardini, A., Zhang, A., and Shiekhattar, R. (2015) Integrator mediates the biogenesis of enhancer RNAs. Nature 525, 399–403 CrossRefMedline

80. Trimarchi, T., Bilal, E., Ntziachristos, P., Fabbri, G., Dalla-Favera, R., Tsigirgos, A., and Atlantis, I. (2014) Genome-wide mapping and characterization of Notch-regulated long noncoding RNAs in acute leukemia. Cell 158, 593–606 CrossRefMedline

81. Wang, K. C., Yang, Y. W., Liu, B., Sanyal, A., Corces-Zimmerman, R., Chen, Y., Lajoie, B. R., Protacio, A., Flynn, R. A., Gupta, R. A., Wysocka, J., Lei, M., Dekker, J., Helms, J. A., and Chang, H. Y. (2011) A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. Nature 472, 120–124 CrossRefMedline

82. Xiang, J. F., Yin, Q. F., Chen, T., Zhang, Y., Zhang, X. O., Wu, Z., Zhang, S., Wang, H. B., Ge, J., Lu, X., Yang, L., and Chen, L. L. (2014) Human colorectal cancer-specific CCAT1-L IncRNA regulates long-range chromatin interactions at the MYC locus. Cell Res. 24, 513–531 CrossRefMedline

83. Vuči´cevi´c, D., Corradin, O., Ntini, E., Scacheri, P. C., and Ørom, U. A. (2015) Long ncRNA expression associates with tissue-specific enhancers. Cell Cycle 14, 253–260 CrossRefMedline

84. Li, W., Notani, D., and Rosenfeld, M. G. (2016) Enhancers as noncoding RNA transcription units: recent insights and future perspectives. Nat. Rev. Genet. 17, 207–223 CrossRefMedline

85. Jonkers, I. and Lis, J. T. (2015) Getting up to speed with transcription elongation by RNA polymerase II. Nat. Rev. Mol. Cell Biol. 16, 167–177 CrossRefMedline

86. Adelman, K., and Lis, J. T. (2012) Promoter-proximal pausing of RNA polymerase II: emerging roles in metazoans. Nat. Rev. Genet. 13, 720–731 CrossRefMedline

87. Hnisz, D., Abraham, B. J., Lee, T. I., Lau, A., Saint-André, V., Sigova, A. A., Hoke, H. A., and Young, R. A. (2013) Super-enhancers in the control of cell identity and disease. Cell 155, 934–947 CrossRefMedline

88. Lovén, J., Hoke, H. A., Lin, C. Y., Lau, A., Orlando, D. A., Vakoc, C. R., Bradner, J. E., Lee, T. I., and Young, R. A. (2013) Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell 153, 320–334 CrossRefMedline

89. Whyte, W. A., Orlando, D. A., Hnisz, D., Abraham, B. J., Lin, C. Y., Kagey, M. H., Rahl, P. B., Lee, T. I., and Young, R. A. (2013) Master transcription factors and mediator establish super-enhancers at key cell identity genes. Cell 153, 307–319 CrossRefMedline

90. Engreitz, J. M., Haines, J. E., Perez, E. M., Munson, G., Chen, J., Kane, M., McDonel, P. E., Guttman, M., and Lander, E. S. (2016) Local regulation of gene expression by lncRNA promoters, transcription and splicing. Nature 539, 452–455 CrossRefMedline

91. Paralkar, V. R., Taborda, C. C., Huang, P., Yao, Y., Kossenkov, A. V., Prasad, R., Luan, J., Davies, J. O., Hughes, J. R., Hardison, R. C., Blobel, G. A., and Weiss, M. J. (2016) Unlinking an lncRNA from its associated cis element. Mol. Cell 62, 104–110 CrossRefMedline