The Hierarchy of Transcriptional Activation: From Enhancer to Promoter

Citation for published version:
Vernimmen, D & Bickmore, WA 2015, 'The Hierarchy of Transcriptional Activation: From Enhancer to Promoter' Trends in Genetics, vol. 31, no. 12, pp. 696-708. DOI: 10.1016/j.tig.2015.10.004

Digital Object Identifier (DOI):
10.1016/j.tig.2015.10.004

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Trends in Genetics

The hierarchy of transcriptional activation: from enhancer to promoter

Douglas Vernimmen[1] & Wendy A. Bickmore[2]

[1]The Roslin Institute, Developmental Biology Division, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, UK. [2]MRC Human Genetics Unit, Institute of Genetics and Molecular Medicine, University of Edinburgh, Edinburgh EH4 2XU, UK.

Corresponding author; Douglas Vernimmen
Telephone +44 (0)131 651 9119
Fax: +44 (0)131 651 9105
e-mail: douglas.vernimmen@roslin.ed.ac.uk

Abstract word count: 65

Characters (including space): 26,895

Words count: 4,160

Figures: 2

Tables: 2

References: 165

Abstract

Regulatory elements (enhancers) that are remote from promoters play a critical role in the spatial, temporal and physiological control of gene expression. Studies on specific loci, together with genome-wide approaches, suggest that there may be many common mechanisms involved in enhancer-promoter communication. Here, we discuss the multi-protein complexes that are recruited to enhancers and the hierarchy of events taking place between regulatory elements and promoters.

Glossary

DNase Hypersenstive Site (DHS): Open region in the genome with increased chromatin accessibility to DNaseI that may reflect the occupation by a transcription factor or the disruption of nucleosome structure. DHS form nucleosome free regions (NFR).

Pioneer Transcription Factors: Transcription factors that can bind their target sites at nucleosomal DNA. This facilitates chromatin remodelling and the binding of other transcription factors with the formation of open chromatin regions (DHS) before enhancer/promoter activation. They also have the property of being retained on mitotic chromosomes and thus could serve as “bookmarking” proteins in mitosis.

Relay Transcription Factors: Transcription factors from the same family (e.g. SOX, GATA) relaying each other for the same binding site (exchange model) as they are expressed at different stages during gene priming.

Transcription Start Site (TSS): Nucleotide marking the site of initiation of mRNA transcription.

Enhancer: Regulatory sequence that increases the rate, or the probability, of transcription of a target gene. An enhancer may lie far away, upstream or downstream from the gene it regulates or may be located in an intron of its target gene or indeed in an intron of another gene.

Locus Control Region (LCR): Genomic region that has the ability to confer physiological levels of tissue-specific expression on a gene linked in *cis*, independent of the gene’s integration site. A LCR can open silent chromatin.

General Transcription Factor (GTF): Also referred to as basal transcription factors (TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH) that bind to core promoters.

Pre-initiation complex (PIC): Association between GTF and RNA polymerase II.

PolII Holocomplex: Complex of subunits forming the complete enzymatically active form of PolII.

Paused PolII: After promoter escape, the engaged PolII is stalled at a pause site, waiting for further signals to progress during elongation.

C-terminal domain (CTD): The C-terminal domain (CTD) of the largest subunit of RNA Polymerase II (PolII) consists of an array of repeats of a heptapeptide sequence (52 repeats in mammals). Amino acids in these repeats are targets for post-translational modification such as phosphorylation of serine 5 (Ser5P) - associated with early elongation (or the paused state of PolII) and Ser2P associated with full elongation.

Activator: *Trans*-acting factor binding a DNA sequence to activate the transcriptional activity of a target gene.

Co-activator: Non-DNA binding protein that associates with an activator and enhances transcription.

Mediator: Large co-activator complex containing 30 subunits in metazoans distributed in three modules: the head, the middle and the tail. Mediator is conserved throughout all eukaryotes.

Integrator: Large co-activator complex containing at least 14 subunits with a total MW over 1MDa. Integrator is restricted to metazoans.

The core promoter

Genes transcribed by RNA Polymerase II (PolII) usually have two distinct families of *cis*-acting elements: the promoter [≤ 1 kb from the transcription start site (TSS)] - composed of a core promoter [1, 2] and nearby (proximal) regulatory elements [3, 4], and more remote (distal) cis-regulatory elements (≥ 1 kb from TSS), which can be enhancers, silencers, insulators or locus control regions (LCR) [3]. The exact composition of core promoter elements may be a key determinant of enhancer-promoter specificity [5] [6]. In mammalian genomes, enhancers are enriched in core promoter elements but are CpG poor whereas promoters are generally CpG rich [7] [8]. Beside the CpG content, enhancers and promoters have broad similarities and overlapping functional properties, and have been considered to form a single class of regulatory element [9].

The core promoter represents the docking site for the General Transcription Factors (GTFs), including TFIIA, TFIIB, TFIID, TFIIE, TFIIF and TFIIH, which, together with PolII, form the pre-initiation complex (PIC) [10]. The PIC is thought to assemble on the core promoter in a specific and sequential order that directs PolII to the nearby TSS [10]. However, this is only sufficient to direct low levels of accurately initiated transcription from DNA templates *in vitro*, a process generally referred to as basal transcription.

The first step in PIC assembly is binding of TFIID, a multi-subunit complex consisting of TATA-box-binding protein (TBP) and a set of 14 TBP-associated factors (TAFs) [10]. Transcription then proceeds through a series of steps, including promoter melting, clearance and escape, before fully functional PolII elongation is achieved. Alternative core promoter complexes may help to maintain specific transcriptional programs in terminally differentiated cell types [11] [12] [13] [14].

Models of transcription regulation view this as a cycle, in which complete PIC assembly is stimulated only once. After PolII escapes from the promoter, TFIID, TFIIE, TFIIH and the Mediator complex (see glossary) remain on the core promoter; subsequent re-initiation then only requires *de novo* recruitment of sub-complexes comprising Pol II-TFIIF and TFIIB (Reviewed in [15]). The various steps of PIC assembly on a core promoter can occur with different timings during differentiation. For example, TBP is already bound to the promoters of α*1-AT*, *HNF-4*α, *VpreB1* and λ*5*, long before differentiation and the transcriptional activation of these genes [16] [17]. Additional transcription factors (TFs) and PolII are recruited later when the genes are transcribed. The one-step recruitment of a (pre-)formed holocomplex (see glossary) at promoters has been also described [18-21]. However, it is worth noting that the right temporal window to appreciate the dynamics of PIC recruitment is often missing from most studies.

In metazoans, the transition from initiation to productive elongation is another important step that involves several levels of regulation. In a region between 30-60 nucleotides downstream the TSS, PolII is often found stalled and thus paused at this site, awaiting additional signals for full elongation [22]. The release of paused PolII is controlled by several TFs such as the negative elongation factor (NELF), the DRB sensitivity-inducing factor (DSIF) and the transcription elongation factor P-TEFb complex (CDK9 and cyclin T). P-TEFb is part of a larger multisubunit complex, called super elongation complex (SEC) [23]. The CTD of PolII plays an important role in elongation by its phosphorylation at several residues (see glossary). Recently, a new multiprotein complex, termed Integrator, has been shown to regulate elongation by recruiting the SEC [24].

Large protein complexes are bound to promoters and enhancers

Transcription is greatly stimulated by a second class of TFs, termed activators. In general, activators are sequence-specific DNA-binding proteins whose recognition sites are usually present near the core promoter and/or at enhancers. Binding of TFs at these elements usually corresponds to nucleosome free regions (NFRs) characterised by hypersensitivity to digestion by nucleases (DNase Hypersensitive Sites, DHS)[2, 25, 26]. This open-chromatin structure can be facilitated by chromatin remodelling factors, which are recruited by TFs and modify histones of the nearby nucleosomes.

Binding of activators does not stimulate transcription from chromatinised templates *in vitro.* The search for factors that stimulate activator-dependent transcription led to the identification of co-activators including; Mediator complexes [27, 28], CBP [29], p300 [30] and BAF [31]. TFs recruit co-activators that can then modify chromatin and/or interact with the core transcription machinery.

The large multiprotein Mediator complex can act as a bridge between transcription activators and components of the PIC [32] (see below). It appears to play important roles in many steps of transcription, including PIC formation and the transition to elongation [32]. Mediator is over a megadalton (MDa) in size and 30nm in length, with distinct structural modules and a flexible structure that changes in response to the binding of different TFs [33]. TF binding seems to induce a conformation change in

Mediator that facilitates PolII binding. Different TFs bind different Mediator subunits, and Mediator complexes that lack a specific subunit can still activate transcription in response to TFs that bind to other subunits. Therefore, among other proteins (e.g. CTCF and cohesin complex) not described in this review, Mediator provides a very important bridge for integrating information coming from different signalling pathways. Mediator might also provide an important binding surface for non-coding RNAs, including eRNAs (see below).

Other co-activators are ATP-dependent chromatin remodelling factors (such as Brahma-associated factor – BAF), or histone acetyltransferases (HAT) – p300/CBP. These can be part of the same complexes. ATP-dependent chromatin-remodelling families form different complexes by a combinatorial assembly of many subunits, to produce biological specificity [34]. BAF complexes, which belong to the SWI/SNF family of ATPase dependent chromatin remodelling complexes, are involved in the relaxation of higher-order chromatin structures and in nucleosome movement and exchange [35]. The p400 SWI/SNF is associated with a HAT (TIP60) in the Tip60/p400 complex that is involved in histone (H2A/H2A.Z) exchange. CREB-binding protein (CBP) and its paralog p300 are co-activator HATs that are found at both promoters and enhancers, and chromatin immunoprecipitation (ChIP) for p300/CBP, together with H3K27ac, is often used to identify active enhancers [36, 37]. However, this is unlikely to be a universal signature of all active enhancers. Indeed, another class of enhancers, containing H4K16ac and KAT8 (MYSM1) but not p300 and H3K27ac have been recently described in embryonic stem (ES) cells [38]. Moreover, HATs also have important non-histone substrates and the role of this in enhancer function is under-studied [39]. Other HATs and HAT-containing complexes (SAGA/PCAF) also have co-activator activity [40].

One of the main questions that needs to be addressed is at which step during gene activation do various nucleoprotein complexes assemble at distant enhancers, and how do these complexes then contribute to promoter accessibility, PIC recruitment and/or assembly, transcription initiation and transcription elongation? Enhancers have been shown to have a role in: PIC recruitment at target promoters [21, 41-45], removing proteasome complexes at promoters [46], the generation of intra-chromosomal loops between regulatory regions [47], and the regulation of elongation [18, 48-51]. Enhancers are also involved in the removal of repressive histone modifications [41, 52-57], suggesting that they also contribute to the delivery of enzymes that regulate histone modifications [58].

Below, we compare studies that have been done in a few mammalian loci in enough depth to provide significant mechanistic insight. Together with supportive genome-wide studies, we discuss if there are common principles that govern the regulation of enhancer-driven transcription.

Sequential recruitment of factors to enhancers and promoters

It is now well established that genes are primed for expression by the binding of pioneer TFs (see glossary) generating nucleosome free regions at regulatory elements and bookmarking the genome for gene expression at a later stage of differentiation [59-63] (Figure 1A). Enhancer priming is followed by the replacement or recruitment of additional TFs ~~(namely *relay*, *tether* and *trigger*, see glossary and below)~~, which may be recruited in a sequential order, mirroring a similar phenomenon on core promoters (e.g. PIC assembly).

Pioneer TFs are able to disrupt chromatin structure and bind to their cognate binding sites irrespective of nucleosomes that may be occluding these sequences (Figure 1A) [64] [65], although this is dependent on the context of other TFs that they associate with in a particular cell type [66, 67]. Pioneer factors, together with chromatin remodelling complexes, are therefore involved in generating an NFR to facilitate the binding of other TFs (Figure 1B). Table 1 lists TFs that have been reported to have pioneer activity. The DNA-binding domain (winged-helix DNA-binding domain/forkhead) of TFs such as HNF3 (FoxA) resembles that of linker histones H1 and H5 and therefore could be involved in chromatin opening by altering nucleosome structure [68]. The CCAAT Box binding factor, NFY has also a core histone-like structure [69] and has been suggested to be involved in opening chromatin by nucleosome replacement [70] [58] and facilitating the binding of master regulators to enhancers in ES cells [71]. It has been suggested that the pioneer activity of PU.1 - a hematopoietic ~~pioneer factor~~ TF - may relate to the tighter DNA-binding of its ETS-domain compared to that of other ETS-family TFs [72]. In reprogramming of somatic cells, Oct4, Sox2, and Klf4 act as pioneer factors, binding at closed chromatin sites [73] [65]. Importantly, their binding occurs first at distal enhancers during early reprogramming steps (Figure 1A), whereas promoter occupancy is a much later event [74] (Figure 1B). The formation of NFRs at promoter and enhancer occur independently from each other. Enhancer priming by pioneer TFs in specific cell-lineages provides a chromatin landscape that can then direct cell-type-specific responses to TFs that act downstream of generic signaling pathways [75-79].

Pioneer TF	**DNA binding domain**	**References**
AP-1	Basic leucine zipper	[76]
AP-2γ (TFAP2C)	Basic helix-span-helix	[80]
FOXA1 (HNF-3α)	Forkhead	[81, 82]

FOXA2 (HNF-3β)	Forkhead	68, 83
FOXE1	Forkhead	84
FOXD3	Forkhead	68, 83
GATA2	2X GATA-type zinc fingers	20
GATA3	2X GATA-type zinc fingers	85
GATA4	2X GATA-type zinc fingers	68, 85, 86
KLF4	3X C2H2-type zinc fingers	73
NF-Y (CBF)	NF-YA/HAP2	70, 71
OCT4	POU-specific + POU-Homeodomain	73 67
OTX2	Homeodomain	67
PAX7	Paired + Homeodomain	87
PBX1	Homeodomain	88
PU.1	Ets	66, 72, 75
SOX2	Hmg box	73, 89, 90
SOX9	Hmg box	91
TP53	p53	92
P63	p53	92
RFX	Rfx-type winged helix	93

Table 1. Pioneer transcription factors involved in DHS formation prior to gene activation.

Pioneer TFs may remain bound throughout the stages of enhancer activation, or they can be replaced by other TFs (exchange model with relay TFs, see glossary) [20, 89, 94]. In some cases, PIC recruitment to enhancers has been reported early during enhancer priming [17, 19], in other cases this is a late event [21]. The first situation led to the idea that enhancers act as a docking site for the recruitment of the general transcription machinery (Figure 1C) that would then be subsequently transferred to the promoter (Figure 1D) [95] [96] [97]. However, many studies have shown that levels of PIC occupancy at enhancers – as judged by ChIP - often appear to be relatively low compared to those at promoters [98]. This could be explained ~~by i) one PIC being spread across the enhancer sequence, which is larger than a core promoter (Figure 1C),~~ i) by the transient nature of several PICs binding to those sequences, or ii) by indirect binding of PICs.

Apparent differences in the timing of recruitment of PIC components to a promoter either before [16, 17, 99], or at the onset of mRNA transcription [18, 21] might be due to the different role(s) attributed to the enhancers, but also to the presence of other important elements located nearby in the proximal promoter. For example, deletion of the Sp1 site at the T-cell receptor beta (TCRβ) promoter, and the CAAT or CACCC boxes at the γ globin promoter, result in failure to detectably recruit TBP at the promoter [100-102], suggesting that these proximal promoter elements are needed to recruit the PIC in order to form a full “promoter complex”.

Transcription from enhancers and promoters

Enhancers are required for transcription of target genes

Genetic ablation is a powerful approach to address how enhancers influence TF and PolII assembly/elongation at promoters. Table 2 summarize the few studies on single loci addressing this, together with the mechanisms of enhancer-promoter communication (see relevant section below). Supporting independent GTFs and PolII recruitment at enhancers and promoters, a few studies have shown that removing a promoter does not affect GTFs or PolII recruitment at the enhancer [21, 103], but removing the enhancer affects GTFs or PolII binding at the promoter [21, 41-44].

Other studies have shown that deletion of enhancers also affects downstream events such as elongation through PolII phosphorylation [18]; [48] [3, 49]. Release of paused PolII might also require additional TFs or additional enhancers [45, 104] (Figure 2C). PolII elongation is regulated by several kinases (cdk7/TFIIH, cdk8/Mediator, and cdk9/p-TEFb) and these are all recruited to genes when expressed [21] [48] and may be delivered through the enhancers [105] [106]. This is consistent with genome-wide studies showing Ser5P [98] and Ser -2P [107, 108] phosphorylated forms of PolII at active enhancers, supporting the idea that enhancers can deliver an activated PolII to target promoters. Alternatively, other regulators of elongation such as DSIF and FACT [49] may be also involved and this idea has been strengthened by recent studies showing the binding of the Integrator at both enhancers and promoters [109]. Overall, the current model arising from these studies is that the promoter is not needed to recruit PolII at enhancers, but the enhancer is always needed to recruit PolII at the promoter or for downstream events such as elongation. Beside these studies, it is worth noting that the timing of an enhancer deletion might also influence outcomes. Enhancers might be required for the initiation of a transcription event whereas others might be important for the maintenance of transcription. For example, histone modifications controlled from a transiently required enhancer might remain after a conditional deletion [110] [111].

Enhancers are also transcribed

Many of the scenarios described above appear to blur the distinction between enhancers and promoters. This is further compounded by evidence of transcription and the production of short RNAs at enhancers (eRNAs) [112] [103, 113] (Figure 1D). The level of expression of these eRNAs is low but positively correlates with the level of mRNA synthesis at nearby genes [103]. eRNAs are short and unstable, probably because the absence of downstream exons (5' splice donors), or the presence of other signals, fails to stabilise the production of the transcribed RNA [114] and indeed the degradation of eRNAs by the exosome is important to prevent the formation of deleterious RNA/DNA hybrids [115]. Note that intragenic enhancers can also function as alternative gene promoters – being spliced to downstream exons to produce stable mRNAs [113]. Conversely, promoters can also work as enhancers [9, 116]. Thus, the distinction between enhancers and promoters lies to some extent simply in their genomic context.

The biological relevance of eRNAs remains controversial as it is still unclear if eRNAs are byproducts of transcription or if they have regulatory functions in themselves. Bidirectional eRNA transcripts can be detected at early stages, prior to the appearance of H3K4me1 at enhancers [79] and to the production of mRNA from the target genes [7] [117] [51, 118, 119]. This could be associated with early recruitment of PolII at enhancers by pioneer TFs [120]. Several attempts at elucidating the role of eRNAs have been addressed by knockdown approaches, showing transcription down-regulation from some [121], [117], [122], [123], [124], [125], but not all, target promoters [118]. Other studies, using a more robust approach, that remove the promoter of the target gene were also not conclusive: in the absence of the *Arc* promoter, eRNA synthesis is abolished, suggesting that it is mRNA dependent [103] [3]; whereas no effect was observed in the same type of experiments on the human growth hormone (*hGH-N*) locus [126]. One study has proposed that eRNAs act as decoy molecules to release NELF from paused PolII at immediate early genes [51]. This is interesting because, this scenario was supported by a recent study showing that the Integrator, is also recruited to enhancers [109]. Integrator is required for the full processing of eRNAs, and depletion of Integrator subunits reduces the production of eRNAs and abolishes enhancer-promoter communication [109]. As Integrator controls the elongation of mRNA transcription of the genes regulated by paused PolII, the role of eRNAs may depend on the context of elongation regulation of as only 50% of genes are regulated by such mechanism [22].

Enhancer-promoter communication

Enhancers can be separated from promoters by distances ranging from a few kilobases to a little over one thousand kilobases [127], yet transcriptional regulation requires some kind of communication between these distant elements (Figure 2). It is still unclear what form this communication takes, e.g. what are the molecules that are transmitted between regulatory element and promoter, when this takes place, and whether this is the same for all classes of enhancers.

Historically, a *linking model* suggested that an activator protein (eg pioneer Figure 1B) first binds the promoter at a proximal sequence and facilitates the recruitment of a second TF to a site located just downstream the former [90, 128, 129]. This cascade of recruitment occurs until it reaches the core promoter to finally recruit the PIC [21, 99].

The *tracking model* and/or a *facilitated tracking model* (Figure 2) is described as a mechanism by which enhancer bound proteins move progressively in an unidirectional manner towards the promoter sometimes without leaving the enhancer sequence, and thus results in the formation of a progressive loop that increases its size until it reaches the promoter to form a stable conformation [99, 130-132] (Figure 2A). In this model, histone acetylation and TF complexes are transiently detected in the intervening sequence and precedes transcription. Originally, it was proposed that intergenic transcripts (eRNAs) are just involved in maintaining an open chromatin structure [133]. Once the gene is expressed, additional transcripts (mostly unidirectional) have been detected across the intervening sequence between enhancers and promoters, which could reflect the tracking of an active PolII [17 43] (Figure 2A).

The looping model implies a direct interaction between two chromosomal regions by looping out the intervening DNA sequence. Various proteins ~~bound at enhancers and promoters~~ have ~~also~~ been proposed to bridge enhancers and promoters together ~~in~~

as looped chromatin structures. These include ~~TFs, such as~~ TAF3 [134], GATA1 [135], EKLF [136], Brg1 [137], Ldb-1 [138], Mediator [139], CTCF [140], SATB1 [141] and cohesins [3, 142-144]. eRNAs have also been proposed to be physically involved in establishing enhancer-promoter 'looping' [124], and involving the Integrator [109].

The stiffness of the chromatin fibre might restrict short-range enhancer-promoter interactions, with a minimal estimated length of 10kb for uninterrupted 30nm chromatin fibres and 0.5kb for naked DNA [145, 146]. NFRs – e.g. created by pioneer TFs - could thus act as hinges, to facilitate chromatin bending and thus the formation of short loops [146 147].

Given the larger distance (> 10 kb and up to 100s of kb) separating many enhancers from their target promoter it is difficult to envisage a mechanism in which the intervening chromatin is directly involved in a mechanism of enhancer-promoter communication such as tracking. Therefore tracking mechanisms are likely limited to enhancers that are close (1-10kb) to their target promoters (Figure 1D). Indeed, the two cases where a facilitated-tracking mechanism has been described, involve a moderate enhancer - promoter distance (Table 2) [99, 132], in comparison to intra-chromosomal looping which has been described for longer enhancer-promoter distances (Table 2) [47, 135, 148, 149]. In the latter cases: do random collisions suffice to facilitate these interactions, or do enhancers actively "seek" for targets both downstream and upstream with equal frequency? Clustered enhancers such as LCRs may be formed by sequential priming progressing from the most upstream element to those downstream, generating ~~a~~ directionality toward the final target promoter [130]. An upstream enhancer (MCS-R2) of the α-globin locus, when relocated downstream of the target genes, still requires interactions with the other upstream enhancers for globin transcription [47] (reviewed in [58]). A polarity between several enhancers has also been shown with the β-globin locus [150]. Deletion of the MCS-R2 α-globin enhancer decreases TF occupancy from the most upstream enhancer towards the downstream promoter [53, 58] again suggesting a directionality in the signal. The duplicated α-globin genes in most species have similar or identical promoters, and it is the gene closest to the upstream elements, that is usually expressed at the higher level (reviewed in [151]). When more than two α-globin genes are present in cis, the additional genes lying downstream are expressed at even lower levels [152]. Thus, it is conceivable that these mechanisms might all be used to regulate a single gene: a looping mechanism between enhancers and promoter for long distance interactions; a tracking mechanism between the different genes of the same cluster, and finally, a linking mechanism between the proximal and the core promoter. Studies using 3C technology and its variations have tended to concentrate attention on long-range interactions, and therefore may have distracted from other possible mechanisms. A study showed that latent enhancers induced by a given stimulus were shown to be frequently at a short distance from target genes [153]; therefore more studies analysing proximal enhancers are needed to characterise the nature of other mechanisms of enhancer-promoter communication.

Concluding Remarks

BOX1: Outstanding questions

- Why PolII recruitment at enhancers sometimes occurs early, long before transcription, and sometimes late, when transcription occurs? In the first scenario, eRNA production might be important for downstream events.

- What is the order of events leading to gene transcription during differentiation or hormonal stimulation, and which feature is cause vs consequence? Molecular dissection of appropriate model loci are required to address these questions.

- Do random collisions suffice to facilitate enhancer-promoter interactions, or do enhancers actively “seek” for targets both downstream and upstream with equal frequency?

- Does increased enhancer-promoter distance in higher organisms favour particular mechanisms of interactions between these elements, e.g. looping rather than tracking or linking?

- What mechanism would a gene use if the intervening DNA sequence is increased, or abolished, or if a ‘linear tracking blocker’ is inserted?

Some genes are regulated by several remote enhancers located at distances that vary from 1Kb to up to 1Mb. There are also genes in clusters that are regulated by the same remote enhancer (e.g. globin genes). Although these genes can be expressed at different stages of development or in different tissues, they are all expressed in the same orientation and their expression level often reduces with increasing distance from the enhancers [44, 151, 152]. During differentiation, enhancers are first primed by pioneer TFs [59], and the signal is subsequently replaced by relay TFs (exchange model). Then, it spreads or loops towards the downstream promoter via other TFs. There is thus a hierarchy among these elements involving a sequential recruitment of TFs, generating the polarity of the transcription signal, from the remote enhancers towards the promoter they regulate. Most of these enhancers have a role as centres of recruitment of PIC. On one hand, the role of the enhancer would be to deliver the PIC to the promoter, and thus explain the enhancer-dependence for PIC recruitment at the promoter. On the other hand, the mechanism(s) that prevent(s) this transfer at the early stages of activation is unclear. Enhancers and promoter seem to communicate by i) physical association and formation of chromosomal loops – in which the intervening DNA sequence would seem to be irrelevant (Looping Model, Figure 2B) or ii) by spreading a signal though the intervening sequence separating enhancer and promoter (Facilitated-Tracking Model) (Figure 2A). Although short distances between these elements are usually found in simpler organisms, the distance has increased in higher organisms. Has this increased distance favoured other mechanisms of interactions between these elements, e.g. looping rather than linking? What mechanism would a gene use if the intervening DNA sequence is increased, or abolished, or if a 'tracking blocker' is inserted. Originally, several studies have addressed the role of a tracking blocker using insulator elements (e.g. [154]). However, the caveats with such experiments, is that we know now that CTCF bound elements are involved in the 3D organisation of the genome in looped structures. Thus, the use of 'linear' tracking blockers such as the lac repressor [155] or *TerF* terminator [156]) would be more appropriate, and only a couple of studies have addressed this [43, 157]. Even for very long-range enhancers, these elements are capable of working at very short distances in enhancer reporter and transgene assays [158]. There are many empty experimental boxes to be filled in Table 2, but we hope this review will help the research community to complete the puzzle. High-throughput sequencing studies have enabled the genome-wide mapping of putative enhancers in diverse cell types. Now functional analyses are required to provide the mechanistic insight into how these enhancers work, and this will be facilitated by genome-editing strategies.

Acknowledgements

We are very grateful to Roger Patient, Bob Hill and Abdenour Soufi for discussions. We apologise to those whose publications we were unable to cite due to space limitations. The Vernimmen lab benefits from funding by the British Society for Haematology (BSH) and the Roslin Foundation. Douglas Vernimmen is supported by a Chancellor's Fellowship at The University of Edinburgh and The Roslin Institute receives Institute Strategic Grant funding from the BBSRC. The Bickmore lab benefits from funding from the UK Medical Research Council and ERC Advanced Grant 249956.

Figure 1. Multi-steps model of long-range gene regulation. **A.** Enhancers are first primed by pioneer transcription factors binding to nucleosomal DNA. **B.** A nucleosome free region (NFR) is formed at an enhancer - often spanning more than one nucleosome. This provides a broad accessible platform for the recruitment of large protein complexes. A similar process occurs independently at the proximal promoter. The *linking model* suggests that an activator protein first binds the promoter at a proximal sequence and facilitates the recruitment of a second TF to a site located just downstream the former. This cascade of recruitment occurs until it reaches the core promoter. This builds a landing platform for the general transcription machinery to the TSS (angled arrow). **C.** The enhancer recruits very large protein complexes, including PIC and Mediator. **D.** The enhancer is now active and is associated with short bi-directional transcripts. Recruitment of PIC at the enhancer can precede that at the promoter, or may happen simultaneously. Proteins and

chromatin structures are drawn approximately to scale. Note that other complexes discussed in the text (e.g. Integrator, BAF, cohesins, etc) are not included for simplification.

Figure 2. Mechanisms of enhancer-promoter communication. A. The facilitated-tracking model is described as a mechanism by which enhancer bound proteins move progressively in an unidirectionally manner towards the promoter, sometimes without leaving the enhancer sequence, and thus results in the formation of a progressive loop that increases its size until it reaches the promoter to form a stable conformation (B). ~~In this model, histone acetylation and TF complexes are transiently detected in the intervening sequence and this precedes transcription~~. The tracking is associated with unidirectional transcripts detected in the intervening DNA sequence. **B.** The looping model implies a direct interaction between two chromosomal regions with the looping out of the intervening DNA sequence. A looped structure together with PolII recruitment at the promoter does not always correlate with transcription, but rather with paused PolII. **C.** Transcription elongation occurs after release of paused PolII, at the onset of looping or afterwards. As in Figure 1, proteins and chromatin structure~~s~~ are drawn approximately to scale.

Locus	**Enhancer (E)**		**Intervening DNA (I)**		**Promoter (P)**		**Proposed Mechanism**	**References**
	HS	**Δ E**	**Size I**	**Δ I**	**Genes**	**Δ P**		
Mouse *Shh*	One HS	√	800kb	x	1	X	Looping	159
Mouse α-globin	MCSR1-4	MCS-R2	20-40kb	x	2	X	Looping	20, 21
Human α-globin	MCSR1-4	MCS-R2 MCSR1-4	30-60kb	x	2	√	Looping	21, 47
Mouse β-globin	HS1-5	HS1-5	40-60kb	x	2	X	Looping	19, 135, 160
Human β-globin	HS1-5	HS2-3			1	√	Looping	161
Human ε-globin	HS-2	x	10kb	√ (Ins)	1	X	Facilitated Tracking	162
Human hGH	HS1-5	HS1	20-35kb	√ (Ter)	5	X	x	42, 43
Human Serpin	HS1-4	?	1-5kb	x	4	X	x	16, 44, 163
Mouse λ5-VpreB1	HS7-9	x	4kb	x	2	X	x	17, 46
Human HNF-4α	One HS	x	6.6kb	x	1	X	Facilitated Tracking	99
Human PSA	One HS	x	4.2kb	x	1	X	Facilitated Tracking	95, 132, 164
Mouse TCRβ	One HS	√	15kb	x	1	√	Facilitated Tracking	41, 101
Human HLA-DRA	One HS	x	2.3kb	x	1	X	x	93, 165
Mouse *Arc* locus	One HS	x	7kb	x	1	√	Looping	51, 103

Table 2. List of loci that have been analysed by deletion (Δ) of Enhancers (E) or Promoters (P) and the mechanisms proposed for these interactions. The number of enhancers (hypersensitive sites, HS) and genes they contain are shown. Note that for the human *ε-globin* and *hGH* genes, the Intervening DNA sequence (I) has been targeted by insertion of an insulator (Ins) or a terminator (Ter) element respectively. Deletion of the promoter of the mouse *TCRβ* gene includes an Sp1 binding site. , Abbreviation: HS: Hypersensitive site. √ : Available, and **x**: no study yet performed.

References

1. Juven-Gershon, T., and Kadonaga, J.T. (2010) Regulation of gene expression via the core promoter and the basal transcriptional machinery. *Dev Biol* 339, 225-229
2. Muller, F., and Tora, L. (2014) Chromatin and DNA sequences in defining promoters for transcription initiation. *Biochim Biophys Acta* 1839, 118-128
3. Ong, C.T., and Corces, V.G. (2011) Enhancer function: new insights into the regulation of tissue-specific gene expression. *Nat Rev Genet* 12, 283-293
4. Lenhard, B.*, et al.* (2012) Metazoan promoters: emerging characteristics and insights into transcriptional regulation. *Nat Rev Genet* 13, 233-245
5. Butler, J.E., and Kadonaga, J.T. (2001) Enhancer-promoter specificity mediated by DPE or TATA core promoter motifs. *Genes Dev* 15, 2515-2519
6. Zabidi, M.A.*, et al.* (2015) Enhancer-core-promoter specificity separates developmental and housekeeping gene regulation. *Nature* 518, 556-559
7. Andersson, R.*, et al.* (2014) An atlas of active enhancers across human cell types and tissues. *Nature* 507, 455-461
8. Andersson, R. (2014) Promoter or enhancer, what's the difference? Deconstruction of established distinctions and presentation of a unifying model. *Bioessays*
9. Andersson, R.*, et al.* (2015) A unified architecture of transcriptional regulatory elements. *Trends Genet* 31, 426-433
10. Sainsbury, S.*, et al.* (2015) Structural basis of transcription initiation by RNA polymerase II. *Nat Rev Mol Cell Biol* 16, 129-143
11. Freiman, R.N.*, et al.* (2001) Requirement of tissue-selective TBP-associated factor TAFII105 in ovarian development. *Science* 293, 2084-2087
12. Muller, F.*, et al.* (2010) Developmental regulation of transcription initiation: more than just changing the actors. *Curr Opin Genet Dev* 20, 533-540
13. Akhtar, W., and Veenstra, G.J. (2011) TBP-related factors: a paradigm of diversity in transcription initiation. *Cell Biosci* 1, 23
14. Herrera, F.J.*, et al.* (2014) Core promoter factor TAF9B regulates neuronal gene expression. *Elife* 3, e02559
15. Maston, G.A.*, et al.* (2006) Transcriptional regulatory elements in the human genome. *Annu Rev Genomics Hum Genet* 7, 29-59
16. Soutoglou, E., and Talianidis, I. (2002) Coordination of PIC assembly and chromatin remodeling during differentiation-induced gene activation. *Science* 295, 1901-1904
17. Szutorisz, H.*, et al.* (2005) Formation of an active tissue-specific chromatin domain initiated by epigenetic marking at the embryonic stem cell stage. *Mol Cell Biol* 25, 1804-1820
18. Sawado, T.*, et al.* (2003) The beta -globin locus control region (LCR) functions primarily by enhancing the transition from transcription initiation to elongation. *Genes Dev* 17, 1009-1018
19. Levings, P.P.*, et al.* (2006) Recruitment of transcription complexes to the beta-globin locus control region and transcription of hypersensitive site 3 prior to erythroid differentiation of murine embryonic stem cells. *FEBS J* 273, 746-755
20. Anguita, E.*, et al.* (2004) Globin gene activation during haemopoiesis is driven by protein complexes nucleated by GATA-1 and GATA-2. *EMBO J* 23, 2841-2852
21. Vernimmen, D.*, et al.* (2007) Long-range chromosomal interactions regulate the timing of the transition between poised and active gene expression. *EMBO J* 26, 2041-2051
22. 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
23. Luo, Z.*, et al.* (2012) The super elongation complex (SEC) family in transcriptional control. *Nat Rev Mol Cell Biol* 13, 543-547
24. Gardini, A.*, et al.* (2014) Integrator regulates transcriptional initiation and pause release following activation. *Mol Cell* 56, 128-139
25. Thurman, R.E.*, et al.* (2012) The accessible chromatin landscape of the human genome. *Nature* 489, 75-82

26. Vierstra, J., *et al.* (2014) Coupling transcription factor occupancy to nucleosome architecture with DNase-FLASH. *Nat Methods* 11, 66-72

27. Thompson, C.M., *et al.* (1993) A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. *Cell* 73, 1361-1375

28. Kim, Y.J., *et al.* (1994) A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Cell* 77, 599-608

29. Naar, A.M., *et al.* (1998) Chromatin, TAFs, and a novel multiprotein coactivator are required for synergistic activation by Sp1 and SREBP-1a in vitro. *Genes Dev* 12, 3020-3031

30. Eckner, R., *et al.* (1994) Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. *Genes Dev* 8, 869-884

31. Wang, W., *et al.* (1996) Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. *EMBO J* 15, 5370-5382

32. Allen, B.L., and Taatjes, D.J. (2015) The Mediator complex: a central integrator of transcription. *Nat Rev Mol Cell Biol* 16, 155-166

33. Tsai, K.L., *et al.* (2014) Subunit architecture and functional modular rearrangements of the transcriptional mediator complex. *Cell* 157, 1430-1444

34. Ho, L., and Crabtree, G.R. (2010) Chromatin remodelling during development. *Nature* 463, 474-484

35. Hargreaves, D.C., and Crabtree, G.R. (2011) ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. *Cell Res* 21, 396-420

36. Creyghton, M.P., *et al.* (2010) Histone H3K27ac separates active from poised enhancers and predicts developmental state. *Proc Natl Acad Sci U S A* 107, 21931-21936

37. Holmqvist, P.H., and Mannervik, M. (2013) Genomic occupancy of the transcriptional co-activators p300 and CBP. *Transcription* 4, 18-23

38. Taylor, G.C., *et al.* (2013) H4K16 acetylation marks active genes and enhancers of embryonic stem cells, but does not alter chromatin compaction. *Genome Res* 23, 2053-2065

39. Chung, H.H., *et al.* (2014) Acetylation at lysine 183 of progesterone receptor by p300 accelerates DNA binding kinetics and transactivation of direct target genes. *J Biol Chem* 289, 2180-2194

40. Lee, K.K., and Workman, J.L. (2007) Histone acetyltransferase complexes: one size doesn't fit all. *Nat Rev Mol Cell Biol* 8, 284-295

41. Spicuglia, S., *et al.* (2002) Promoter activation by enhancer-dependent and -independent loading of activator and coactivator complexes. *Mol Cell* 10, 1479-1487

42. Ho, Y., *et al.* (2002) A defined locus control region determinant links chromatin domain acetylation with long-range gene activation. *Mol Cell* 9, 291-302

43. Ho, Y., *et al.* (2006) Locus control region transcription plays an active role in long-range gene activation. *Mol Cell* 23, 365-375

44. Zhao, H., *et al.* (2007) The locus control region activates serpin gene expression through recruitment of liver-specific transcription factors and RNA polymerase II. *Mol Cell Biol* 27, 5286-5295

45. Ghavi-Helm, Y., *et al.* (2014) Enhancer loops appear stable during development and are associated with paused polymerase. *Nature*

46. Szutorisz, H., *et al.* (2006) The proteasome restricts permissive transcription at tissue-specific gene loci in embryonic stem cells. *Cell* 127, 1375-1388

47. Vernimmen, D., *et al.* (2009) Chromosome looping at the human alpha-globin locus is mediated via the major upstream regulatory element (HS -40). *Blood* 114, 4253-4260

48. Song, S.H., *et al.* (2010) Multiple functions of Ldb1 required for beta-globin activation during erythroid differentiation. *Blood* 116, 2356-2364

49. Bender, M.A., *et al.* (2012) The hypersensitive sites of the murine beta-globin locus control region act independently to affect nuclear localization and transcriptional elongation. *Blood* 119, 3820-3827

50. Lin, C., *et al.* (2013) The RNA Pol II elongation factor Ell3 marks enhancers in ES cells and primes future gene activation. *Cell* 152, 144-156

51. Schaukowitch, K., *et al.* (2014) Enhancer RNA facilitates NELF release from immediate early genes. *Mol Cell* 56, 29-42
52. Seenundun, S., *et al.* (2010) UTX mediates demethylation of H3K27me3 at muscle-specific genes during myogenesis. *EMBO J* 29, 1401-1411
53. Vernimmen, D., *et al.* (2011) Polycomb eviction as a new distant enhancer function. *Genes Dev* 25, 1583-1588
54. Taberlay, P.C., *et al.* (2011) Polycomb-repressed genes have permissive enhancers that initiate reprogramming. *Cell* 147, 1283-1294
55. Williams, K., *et al.* (2014) The Histone Lysine Demethylase JMJD3/KDM6B Is Recruited to p53 Bound Promoters and Enhancer Elements in a p53 Dependent Manner. *PLoS One* 9, e96545
56. Kondo, T., *et al.* (2014) Polycomb potentiates meis2 activation in midbrain by mediating interaction of the promoter with a tissue-specific enhancer. *Dev Cell* 28, 94-101
57. Park, D.H., *et al.* (2014) Activation of neuronal gene expression by the JMJD3 demethylase is required for postnatal and adult brain neurogenesis. *Cell Rep* 8, 1290-1299
58. Vernimmen, D. (2014) Uncovering enhancer functions using the alpha-globin locus. *PLoS Genet* 10, e1004668
59. Zaret, K.S., and Carroll, J.S. (2011) Pioneer transcription factors: establishing competence for gene expression. *Genes Dev* 25, 2227-2241
60. Kadauke, S., *et al.* (2012) Tissue-specific mitotic bookmarking by hematopoietic transcription factor GATA1. *Cell* 150, 725-737
61. Caravaca, J.M., *et al.* (2013) Bookmarking by specific and nonspecific binding of FoxA1 pioneer factor to mitotic chromosomes. *Genes Dev* 27, 251-260
62. Kadauke, S., and Blobel, G.A. (2013) Mitotic bookmarking by transcription factors. *Epigenetics Chromatin* 6, 6
63. Rada-Iglesias, A. (2013) Pioneering barren land: mitotic bookmarking by transcription factors. *Dev Cell* 24, 342-344
64. Sherwood, R.I., *et al.* (2014) Discovery of directional and nondirectional pioneer transcription factors by modeling DNase profile magnitude and shape. *Nat Biotechnol* 32, 171-178
65. Soufi, A., *et al.* (2015) Pioneer transcription factors target partial DNA motifs on nucleosomes to initiate reprogramming. *Cell* 161, 555-568
66. Heinz, S., *et al.* (2010) Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell* 38, 576-589
67. Buecker, C., *et al.* (2014) Reorganization of enhancer patterns in transition from naive to primed pluripotency. *Cell Stem Cell* 14, 838-853
68. Cirillo, L.A., *et al.* (2002) Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. *Mol Cell* 9, 279-289
69. Romier, C., *et al.* (2003) The NF-YB/NF-YC structure gives insight into DNA binding and transcription regulation by CCAAT factor NF-Y. *J Biol Chem* 278, 1336-1345
70. Gatta, R., and Mantovani, R. (2008) NF-Y substitutes H2A-H2B on active cell-cycle promoters: recruitment of CoREST-KDM1 and fine-tuning of H3 methylations. *Nucleic Acids Res* 36, 6592-6607
71. Oldfield, A.J., *et al.* (2014) Histone-fold domain protein NF-Y promotes chromatin accessibility for cell type-specific master transcription factors. *Mol Cell* 55, 708-722
72. Wang, S., *et al.* (2014) Mechanistic Heterogeneity in Site Recognition by the Structurally Homologous DNA-Binding Domains of the ETS-Family Transcription Factors Ets-1 and PU.1. *J Biol Chem*
73. Soufi, A., *et al.* (2012) Facilitators and impediments of the pluripotency reprogramming factors' initial engagement with the genome. *Cell* 151, 994-1004
74. Soufi, A., and Zaret, K.S. (2013) Understanding impediments to cellular conversion to pluripotency by assessing the earliest events in ectopic transcription factor binding to the genome. *Cell Cycle* 12, 1487-1491
75. Ghisletti, S., *et al.* (2010) Identification and characterization of enhancers controlling the inflammatory gene expression program in macrophages. *Immunity* 32, 317-328

76. Biddie, S.C., *et al.* (2011) Transcription factor AP1 potentiates chromatin accessibility and glucocorticoid receptor binding. *Mol Cell* 43, 145-155

77. Trompouki, E., *et al.* (2011) Lineage regulators direct BMP and Wnt pathways to cell-specific programs during differentiation and regeneration. *Cell* 147, 577-589

78. Samstein, R.M., *et al.* (2012) Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. *Cell* 150, 29-38

79. Kaikkonen, M.U., *et al.* (2013) Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription. *Mol Cell* 51, 310-325

80. Tan, S.K., *et al.* (2011) AP-2gamma regulates oestrogen receptor-mediated long-range chromatin interaction and gene transcription. *EMBO J* 30, 2569-2581

81. Cirillo, L.A., *et al.* (1998) Binding of the winged-helix transcription factor HNF3 to a linker histone site on the nucleosome. *EMBO J* 17, 244-254

82. Serandour, A.A., *et al.* (2011) Epigenetic switch involved in activation of pioneer factor FOXA1-dependent enhancers. *Genome Res* 21, 555-565

83. Xu, J., *et al.* (2009) Transcriptional competence and the active marking of tissue-specific enhancers by defined transcription factors in embryonic and induced pluripotent stem cells. *Genes Dev* 23, 2824-2838

84. Cuesta, I., *et al.* (2007) The forkhead factor FoxE1 binds to the thyroperoxidase promoter during thyroid cell differentiation and modifies compacted chromatin structure. *Mol Cell Biol* 27, 7302-7314

85. Shoemaker, J., *et al.* (2006) GATA-3 directly remodels the IL-10 locus independently of IL-4 in CD4+ T cells. *J Immunol* 176, 3470-3479

86. Miranda-Carboni, G.A., *et al.* (2011) GATA4 regulates estrogen receptor-alpha-mediated osteoblast transcription. *Mol Endocrinol* 25, 1126-1136

87. Budry, L., *et al.* (2012) The selector gene Pax7 dictates alternate pituitary cell fates through its pioneer action on chromatin remodeling. *Genes Dev* 26, 2299-2310

88. Berkes, C.A., *et al.* (2004) Pbx marks genes for activation by MyoD indicating a role for a homeodomain protein in establishing myogenic potential. *Mol Cell* 14, 465-477

89. Liber, D., *et al.* (2010) Epigenetic priming of a pre-B cell-specific enhancer through binding of Sox2 and Foxd3 at the ESC stage. *Cell Stem Cell* 7, 114-126

90. Chen, J., *et al.* (2014) Single-molecule dynamics of enhanceosome assembly in embryonic stem cells. *Cell* 156, 1274-1285

91. Adam, R.C., *et al.* (2015) Pioneer factors govern super-enhancer dynamics in stem cell plasticity and lineage choice. *Nature*

92. Sammons, M.A., *et al.* (2015) TP53 engagement with the genome occurs in distinct local chromatin environments via pioneer factor activity. *Genome Res*

93. Masternak, K., *et al.* (2003) Chromatin remodeling and extragenic transcription at the MHC class II locus control region. *Nat Immunol* 4, 132-137

94. Dillon, N. (2012) Factor mediated gene priming in pluripotent stem cells sets the stage for lineage specification. *Bioessays* 34, 194-204

95. Louie, M.C., *et al.* (2003) Androgen-induced recruitment of RNA polymerase II to a nuclear receptor-p160 coactivator complex. *Proc Natl Acad Sci U S A* 100, 2226-2230

96. Koch, F., *et al.* (2008) Genome-wide RNA polymerase II: not genes only! *Trends Biochem Sci* 33, 265-273

97. Stumpf, M., *et al.* (2010) Specific erythroid-lineage defect in mice conditionally deficient for Mediator subunit Med1. *Proc Natl Acad Sci U S A* 107, 21541-21546

98. Koch, F., *et al.* (2011) Transcription initiation platforms and GTF recruitment at tissue-specific enhancers and promoters. *Nat Struct Mol Biol* 18, 956-963

99. Hatzis, P., and Talianidis, I. (2002) Dynamics of enhancer-promoter communication during differentiation-induced gene activation. *Mol Cell* 10, 1467-1477

100. Fang, X., *et al.* (2004) Developmentally specific role of the CCAAT box in regulation of human gamma-globin gene expression. *J Biol Chem* 279, 5444-5449

101. Oestreich, K.J., *et al.* (2006) Regulation of TCRbeta gene assembly by a promoter/enhancer holocomplex. *Immunity* 24, 381-391

102. Li, Q., *et al.* (2006) Transcriptional potential of the gamma-globin gene is dependent on the CACCC box in a developmental stage-specific manner. *Nucleic Acids Res* 34, 3909-3916

103. Kim, T.K., *et al.* (2010) Widespread transcription at neuronal activity-regulated enhancers. *Nature* 465, 182-187

104. Jin, F., *et al.* (2013) A high-resolution map of the three-dimensional chromatin interactome in human cells. *Nature* 503, 290-294

105. Stadhouders, R., *et al.* (2012) Dynamic long-range chromatin interactions control Myb proto-oncogene transcription during erythroid development. *EMBO J* 31, 986-999

106. Loven, J., *et al.* (2013) Selective inhibition of tumor oncogenes by disruption of super-enhancers. *Cell* 153, 320-334

107. Zentner, G.E., *et al.* (2011) Epigenetic signatures distinguish multiple classes of enhancers with distinct cellular functions. *Genome Res* 21, 1273-1283

108. Zhang, W., *et al.* (2012) Bromodomain-containing protein 4 (BRD4) regulates RNA polymerase II serine 2 phosphorylation in human CD4+ T cells. *J Biol Chem* 287, 43137-43155

109. Lai, F., *et al.* (2015) Integrator mediates the biogenesis of enhancer RNAs. *Nature*

110. Sen, R., and Grosschedl, R. (2010) Memories of lost enhancers. *Genes Dev* 24, 973-979

111. Anamika, K., *et al.* (2010) Lessons from genome-wide studies: an integrated definition of the coactivator function of histone acetyl transferases. *Epigenetics Chromatin* 3, 18

112. De Santa, F., *et al.* (2010) A large fraction of extragenic RNA pol II transcription sites overlap enhancers. *PLoS Biol* 8, e1000384

113. Kowalczyk, M.S., *et al.* (2012) Intragenic enhancers act as alternative promoters. *Mol Cell* 45, 447-458

114. Core, L.J., *et al.* (2014) Analysis of nascent RNA identifies a unified architecture of initiation regions at mammalian promoters and enhancers. *Nat Genet* 46, 1311-1320

115. Pefanis, E., *et al.* (2015) RNA exosome-regulated long non-coding RNA transcription controls super-enhancer activity. *Cell* 161, 774-789

116. Li, G., *et al.* (2012) Extensive promoter-centered chromatin interactions provide a topological basis for transcription regulation. *Cell* 148, 84-98

117. Li, W., *et al.* (2013) Functional roles of enhancer RNAs for oestrogen-dependent transcriptional activation. *Nature* 498, 516-520

118. Hah, N., *et al.* (2013) Enhancer transcripts mark active estrogen receptor binding sites. *Genome Res* 23, 1210-1223

119. Arner, E., *et al.* (2015) Gene regulation. Transcribed enhancers lead waves of coordinated transcription in transitioning mammalian cells. *Science* 347, 1010-1014

120. Hsu, H.T., *et al.* (2015) TRANSCRIPTION. Recruitment of RNA polymerase II by the pioneer transcription factor PHA-4. *Science* 348, 1372-1376

121. Lam, M.T., *et al.* (2013) Rev-Erbs repress macrophage gene expression by inhibiting enhancer-directed transcription. *Nature* 498, 511-515

122. Melo, C.A., *et al.* (2013) eRNAs are required for p53-dependent enhancer activity and gene transcription. *Mol Cell* 49, 524-535

123. Mousavi, K., *et al.* (2013) eRNAs promote transcription by establishing chromatin accessibility at defined genomic loci. *Mol Cell* 51, 606-617

124. Hsieh, C.L., *et al.* (2014) Enhancer RNAs participate in androgen receptor-driven looping that selectively enhances gene activation. *Proc Natl Acad Sci U S A* 111, 7319-7324

125. Ilott, N.E., *et al.* (2014) Long non-coding RNAs and enhancer RNAs regulate the lipopolysaccharide-induced inflammatory response in human monocytes. *Nat Commun* 5, 3979

126. Yoo, E.J., *et al.* (2012) An RNA-independent linkage of noncoding transcription to long-range enhancer function. *Mol Cell Biol* 32, 2020-2029

127. Noonan, J.P., and McCallion, A.S. (2010) Genomics of long-range regulatory elements. *Annu Rev Genomics Hum Genet* 11, 1-23

128. Bulger, M., and Groudine, M. (1999) Looping versus linking: toward a model for long-distance gene activation. *Genes Dev* 13, 2465-2477

129. Dorsett, D. (1999) Distant liaisons: long-range enhancer-promoter interactions in Drosophila. *Curr Opin Genet Dev* 9, 505-514

130. Blackwood, E.M., and Kadonaga, J.T. (1998) Going the distance: a current view of enhancer action. *Science* 281, 60-63

131. Tuan, D.*, et al.* (1992) Transcription of the hypersensitive site HS2 enhancer in erythroid cells. *Proc Natl Acad Sci U S A* 89, 11219-11223

132. Wang, Q.*, et al.* (2005) Spatial and temporal recruitment of androgen receptor and its coactivators involves chromosomal looping and polymerase tracking. *Mol Cell* 19, 631-642

133. Routledge, S.J., and Proudfoot, N.J. (2002) Definition of transcriptional promoters in the human beta globin locus control region. *J Mol Biol* 323, 601-611

134. Liu, Z.*, et al.* (2011) Control of embryonic stem cell lineage commitment by core promoter factor, TAF3. *Cell* 146, 720-731

135. Vakoc, C.R.*, et al.* (2005) Proximity among distant regulatory elements at the beta-globin locus requires GATA-1 and FOG-1. *Mol Cell* 17, 453-462

136. Drissen, R.*, et al.* (2004) The active spatial organization of the beta-globin locus requires the transcription factor EKLF. *Genes Dev* 18, 2485-2490

137. Kim, S.I.*, et al.* (2009) BRG1 requirement for long-range interaction of a locus control region with a downstream promoter. *Proc Natl Acad Sci U S A* 106, 2259-2264

138. Song, S.H.*, et al.* (2007) A positive role for NLI/Ldb1 in long-range beta-globin locus control region function. *Mol Cell* 28, 810-822

139. Kagey, M.H.*, et al.* (2010) Mediator and cohesin connect gene expression and chromatin architecture. *Nature* 467, 430-435

140. Mishiro, T.*, et al.* (2009) Architectural roles of multiple chromatin insulators at the human apolipoprotein gene cluster. *EMBO J* 28, 1234-1245

141. Cai, S.*, et al.* (2006) SATB1 packages densely looped, transcriptionally active chromatin for coordinated expression of cytokine genes. *Nat Genet* 38, 1278-1288

142. Nativio, R.*, et al.* (2009) Cohesin is required for higher-order chromatin conformation at the imprinted IGF2-H19 locus. *PLoS Genet* 5, e1000739

143. Hadjur, S.*, et al.* (2009) Cohesins form chromosomal cis-interactions at the developmentally regulated IFNG locus. *Nature* 460, 410-413

144. Hou, C.*, et al.* (2010) Cell type specificity of chromatin organization mediated by CTCF and cohesin. *Proc Natl Acad Sci U S A* 107, 3651-3656

145. Rippe, K. (2001) Making contacts on a nucleic acid polymer. *Trends Biochem Sci* 26, 733-740

146. Gondor, A., and Ohlsson, R. (2009) Chromosome crosstalk in three dimensions. *Nature* 461, 212-217

147. Bickmore, W.A. (2013) The spatial organization of the human genome. *Annu Rev Genomics Hum Genet* 14, 67-84

148. Palstra, R.J.*, et al.* (2003) The beta-globin nuclear compartment in development and erythroid differentiation. *Nat Genet* 35, 190-194

149. Jing, H.*, et al.* (2008) Exchange of GATA factors mediates transitions in looped chromatin organization at a developmentally regulated gene locus. *Mol Cell* 29, 232-242

150. Tanimoto, K.*, et al.* (1999) Effects of altered gene order or orientation of the locus control region on human beta-globin gene expression in mice. *Nature* 398, 344-348

151. Higgs, D.R.*, et al.* (1989) A review of the molecular genetics of the human alpha-globin gene cluster. *Blood* 73, 1081-1104

152. Vestri, R.*, et al.* (1994) Expression gradient in sheep alpha alpha and alpha alpha alpha globin gene haplotypes: mRNA levels. *Blood* 83, 2317-2322

153. Ostuni, R.*, et al.* (2013) Latent enhancers activated by stimulation in differentiated cells. *Cell* 152, 157-171

154. Rollins, R.A.*, et al.* (1999) Nipped-B, a Drosophila homologue of chromosomal adherins, participates in activation by remote enhancers in the cut and Ultrabithorax genes. *Genetics* 152, 577-593

155. Lee, J., and Goldfarb, A. (1991) lac repressor acts by modifying the initial transcribing complex so that it cannot leave the promoter. *Cell* 66, 793-798

156. Dye, M.J., and Proudfoot, N.J. (1999) Terminal exon definition occurs cotranscriptionally and promotes termination of RNA polymerase II. *Mol Cell* 3, 371-378

157. Ling, J.*, et al.* (2004) HS2 enhancer function is blocked by a transcriptional terminator inserted between the enhancer and the promoter. *J Biol Chem* 279, 51704-51713

158. Anderson, E.*, et al.* (2014) Mapping the Shh long-range regulatory domain. *Development* 141, 3934-3943

159. Amano, T.*, et al.* (2009) Chromosomal dynamics at the Shh locus: limb bud-specific differential regulation of competence and active transcription. *Dev Cell* 16, 47-57

160. Johnson, K.D.*, et al.* (2003) Highly restricted localization of RNA polymerase II within a locus control region of a tissue-specific chromatin domain. *Mol Cell Biol* 23, 6484-6493

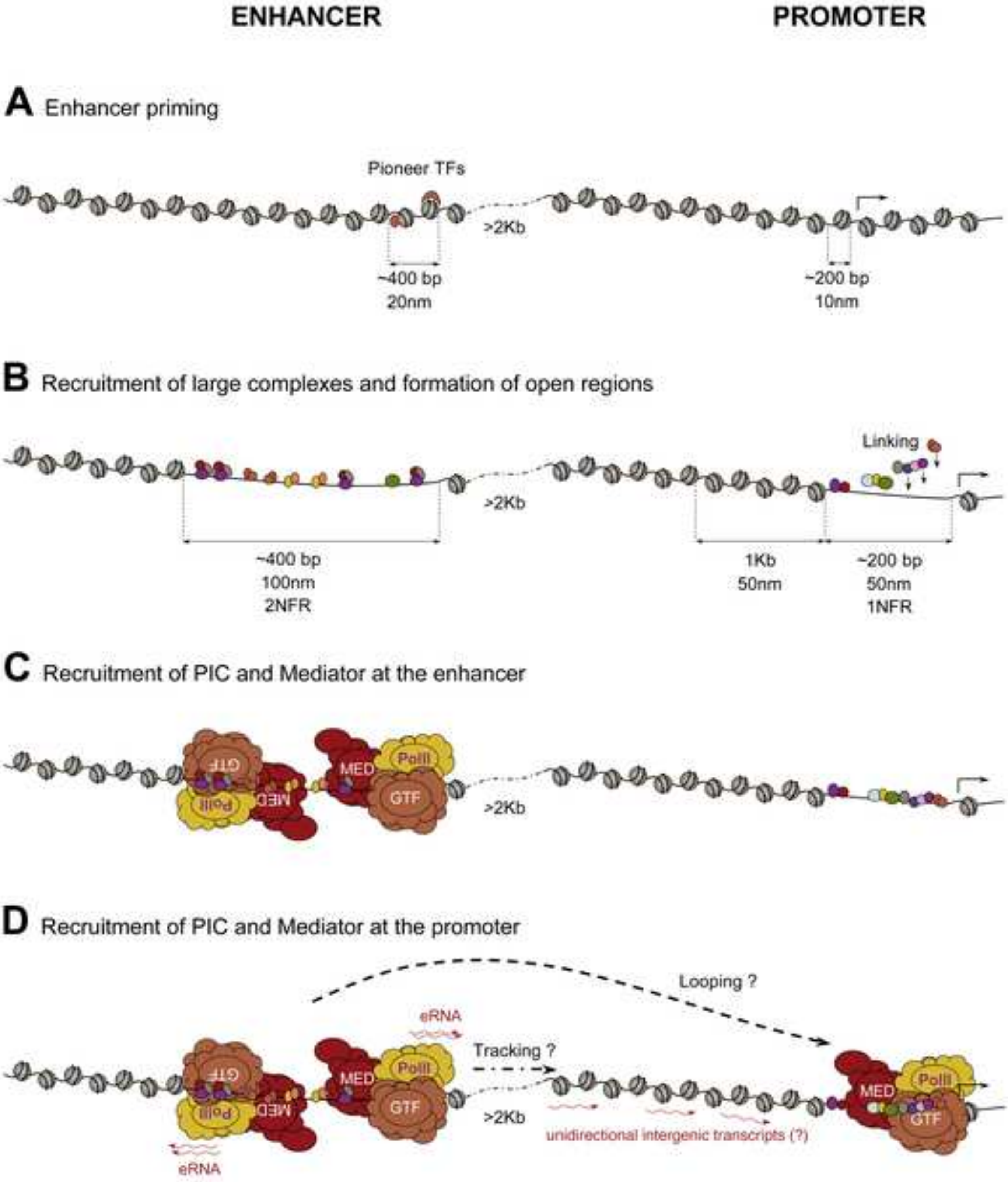
161. Patrinos, G.P.*, et al.* (2004) Multiple interactions between regulatory regions are required to stabilize an active chromatin hub. *Genes Dev* 18, 1495-1509

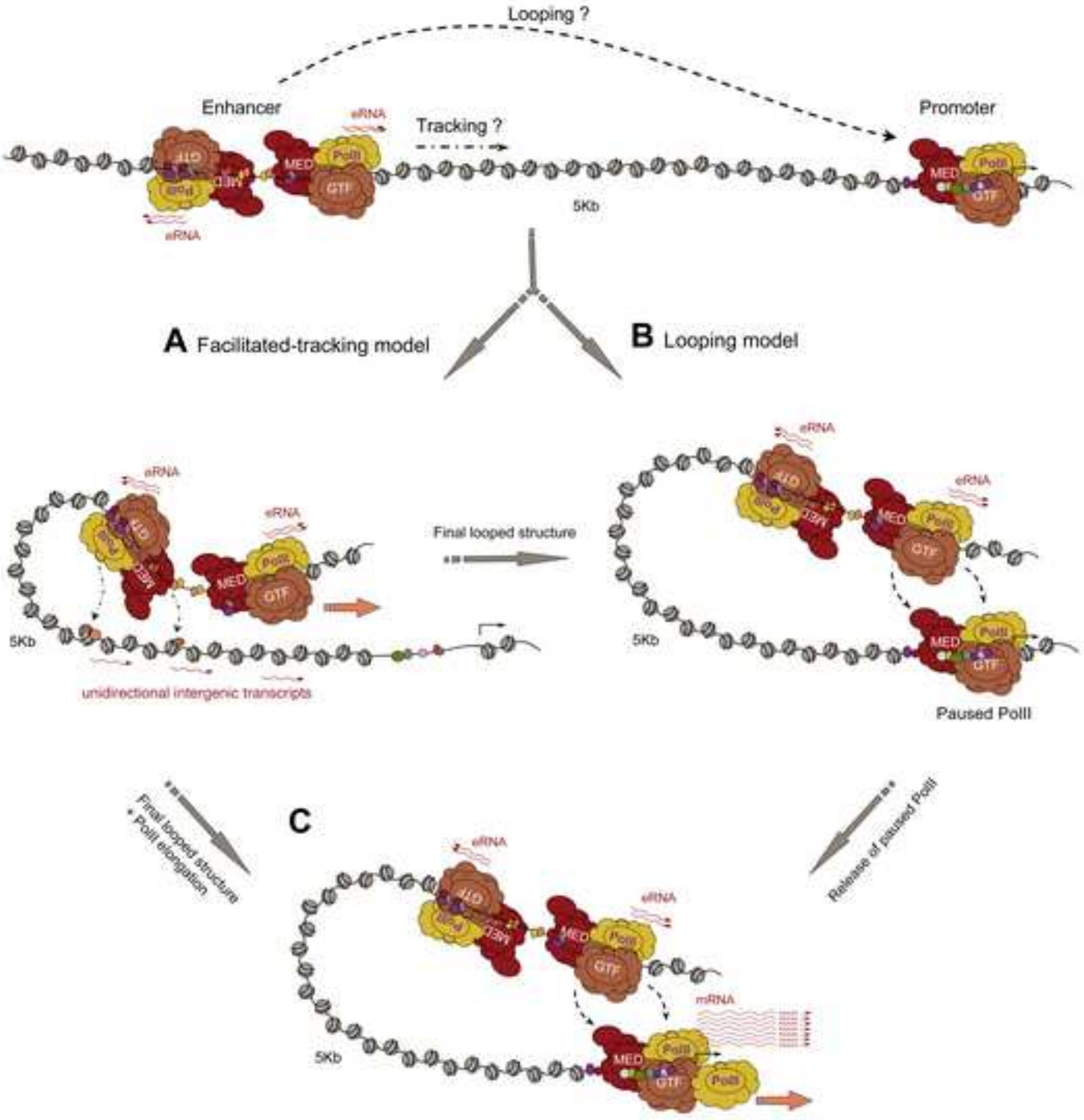
162. Zhu, X.*, et al.* (2007) A facilitated tracking and transcription mechanism of long-range enhancer function. *Nucleic Acids Res* 35, 5532-5544

163. Marsden, M.D., and Fournier, R.E. (2003) Chromosomal elements regulate gene activity and chromatin structure of the human serpin gene cluster at 14q32.1. *Mol Cell Biol* 23, 3516-3526

164. Shang, Y.*, et al.* (2002) Formation of the androgen receptor transcription complex. *Mol Cell* 9, 601-610

165. Spilianakis, C.*, et al.* (2003) CIITA regulates transcription onset viaSer5-phosphorylation of RNA Pol II. *EMBO J* 22, 5125-5136


ENHANCER
PROMOTER
A Enhancer priming
Pioneer TFs
>2Kb
~400 bp
20nm
~200 bp
10nm
B Recruitment of large complexes and formation of open regions
Linking
>2Kb
~400 bp
100nm
2NFR
1Kb
50nm
~200 bp
50nm
1NFR
C Recruitment of PIC and Mediator at the enhancer
GTF
PolII
MED
MED
PolII
GTF
>2Kb
D Recruitment of PIC and Mediator at the promoter
Looping ?
eRNA
Tracking ?
GTF
PolII
MED
MED
PolII
GTF
>2Kb
eRNA
unidirectional intergenic transcripts (?)
MED
PolII
GTF


Looping ?
Enhancer
eRNA
Tracking ?
Promoter
GTF
MED
PolII
eRNA
5Kb
A Facilitated-tracking model
B Looping model
eRNA
eRNA
Final looped structure
eRNA
eRNA
MED
PolII
GTF
5Kb
unidirectional intergenic transcripts
5Kb
MED
PolII
GTF
Paused PolII
Final looped structure + PolII elongation
Release of paused PolII
C
eRNA
eRNA
MED
PolII
GTF
mRNA
5Kb
MED
PolII
GTF
PolII
Full elongation

The hierarchy of transcriptional activation: from enhancer to promoter

Douglas Vernimmen & Wendy A. Bickmore

Trends Box:

- Enhancers are first primed by *pioneer* transcription factors.

- Other transcription factors are likely required for subsequent events.

- There is a hierarchy between enhancers and the promoters that they regulate.

- Enhancers and promoters share similar properties, but differ in the characteristics and the abundance of the RNAs that they produce.

- By recruiting the pre-initiation complex and other proteins, enhancers have a role of increasing the concentration of the transcription machinery at target promoters.

The hierarchy of transcriptional activation: from enhancer to promoter

Douglas Vernimmen & Wendy A. Bickmore

BOX1: Outstanding questions

- Why PolII recruitment at enhancers sometimes occurs early, long before transcription, and sometimes late, when transcription occurs? In the first scenario, eRNA production might be important for downstream events.

- What is the order of events leading to gene transcription during differentiation or hormonal stimulation, and which feature is cause vs consequence? Molecular dissection of appropriate model loci are required to address these questions.

- Do random collisions suffice to facilitate enhancer-promoter interactions, or do enhancers actively “seek” for targets both downstream and upstream with equal frequency?

- Does increased enhancer-promoter distance in higher organisms favour particular mechanisms of interactions between these elements, e.g. looping rather than tracking or linking?

- What mechanism would a gene use if the intervening DNA sequence is increased, or abolished, or if a ‘linear tracking blocker’ is inserted?