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Regulatory enhancer–core-promoter communication via transcription factors and cofactors

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Abstract

Gene expression is regulated by genomic enhancers that recruit transcription factors and cofactors to activate transcription from target core-promoters. Over the past years, thousands of enhancers and core-promoters in animal genomes have been annotated and we learned much about the domain-structure in which animal regulatory genomes are organized. Enhancer – core-promoter targeting occurs at several levels, including regulatory domains, DNA accessibility and sequence-encoded core-promoter specificities that are likely mediated by different regulatory proteins. Here, we review our current knowledge about enhancer – core-promoter targeting, regulatory communication between enhancers and core-promoters, and the protein factors involved. We conclude with an outlook on open questions that we find particularly interesting and will likely lead to additional insights in the upcoming years.

Keywords

Core-promoters; enhancers; enhancer–core-promoter specificity; transcription regulation; transcription factors; cofactors

Gene expression and its spatio-temporal regulation is central to the development and adult physiology in all multicellular organisms. It enables formation of distinct cell types with specialized morphologies and functions by allowing different genes to be activated in a cell-type-specific manner. Transcription, the copying of a gene from genomic DNA into RNA, constitutes the first and one of the most heavily regulated steps of gene expression. Indeed, the RNA content of different cell types differs greatly and correlates well with protein abundance for many genes [1]. Furthermore, mis-regulation at the level of transcription underlies several developmental disorders and diseases such as cancer [2,3]. Transcription initiates within **core-promoters** (See Glossary), short sequences of around 100 base pairs (bps) surrounding the transcription start sites (TSSs) at the 5’ start of genes. Core-promoters recruit Pol II, assemble the Pre-Initiation Complex (PIC) and dictate the accurate position of initiation and direction of transcription [4]. Typically however, core-promoters on their own cannot support efficient transcription and exhibit only low basal activities. Instead, their cell-type-specific activities are typically determined by enhancers, the second key type of transcriptional regulatory elements [5,6].

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Enhancers are genomic DNA elements of up to several hundred bps in length, which contain short transcription factor (TF) recognition sequences or binding sites. Through these sites, combinations of TFs are recruited to enhancers and in turn recruit cofactors with a variety of biochemical functions (Figure 1, Key Figure). Through the combined activating or repressive cues of the different TFs and cofactors, enhancers exert their overall regulatory function to control transcription from target core-promoters irrespective of their orientation and distance [7]. Since enhancers can act over short and long distances, i.e. their positions with respect to their target core-promoters can be arbitrary, they do not always regulate the nearest gene.

An important question in biology is how activating regulatory cues are communicated from enhancers to their correct target core-promoters. We discuss different modes of specifying enhancer–core-promoter communication, starting with the organization of the regulatory genome into chromatin domains, followed by the specification of enhancer – core-promoter contacts by DNA accessibility and enhancer–core-promoter tethering, and finally how enhancer and core-promoter sequences recruit different regulatory proteins that mediate the regulatory communication and specificity.

Core-promoter Targeting within Topologically Associating Domains (TADs)

Animal *cis*-regulatory genomes are organized locally into domains that can be several kilobases or megabases in length (Figure 2A). These domains are typically delineated by insulator proteins such as CTCF (reviewed in [8–10]) or by broadly expressed housekeeping genes [11–13]. Within these topologically associating domains (TADs; refs. [12,14,15]), chromatin contacts are more frequent than elsewhere, as measured by chromosome conformation capture (3C) and variant techniques [16–22] or by fluorescence *in situ* hybridization (FISH) [23].

Ample evidence supports a role of TADs in restricting or directing enhancer function during transcriptional regulation: for example, TAD boundaries and insulator binding are depleted between enhancers and their target core-promoters [24], and TAD boundaries curb the spreading of chromatin marks associated with transcriptional activity [12,25–27]. Moreover, while enhancers function generally independently of their orientation, enhancers with proximal CTCF binding sites can show directional activities *in vivo* that can be inversed by inverting the DNA fragment containing the enhancer and CTCF binding site [28]. Within TADs, individual enhancers are able to activate reporter genes irrespective of the positions into which the reporters were integrated, suggesting that enhancer – core-promoter communication within TADs it not restricted to specific positions [29,30].

Furthermore, disruptions of TAD boundaries lead to gene deregulation, manifesting in conditions such as polydactylies in human patients and in mouse models [31]. In addition, chromosomal rearrangements [32] or reduced CTCF binding due to the hypermethylation of CTCF binding sites [33] can impair boundary function and have been implicated in cancer. Such alterations create new enhancer–core-promoter interactions, leading to gene mis-expression and increase in cancer cell oncogenicity (reviewed in [34]), and indeed,

mutations of CTCF sites are enriched in cancer associated SNPs [35]. Together, these observations suggest that TADs act as gene regulatory units.

Core-promoter Selection via DNA Accessibility and Tethering

While genes within TADs are indeed often coordinately regulated [36], many genes are not co-expressed, and enhancers and their target core-promoters are not necessarily adjacent and collinear in the genomic sequence. For example, during *Drosophila* embryogenesis, the neighboring genes *Sex combs reduced* (*Scr*) and *fushi tarazu* (*ftz*) are expressed in different patterns and their enhancers are not collinear (Figure 2B): the enhancer of *Scr* is located 3' of the *ftz* gene and the enhancer of *ftz* lies between the two genes. The selectivity of the *Scr* enhancer for the *Scr* promoter can be recapitulated in reporter assays and depends on a promoter-proximal tethering element (PTE) [37] that might mediate specific enhancer – core-promoter spatial proximities or contacts. Interestingly, another PTE identified at the Bithorax complex locus even enables enhancer communication across insulators [38,39]. Equivalent sequences have also been described within or next to enhancers, which mediated the enhancers' contacts to their target promoter, thus termed promoter targeting sequences [40]. Such enhancer – core-promoter tethering would be compatible with the observation of looping and stable enhancer-Polymerase contacts as observed during development [41]. In experimental setups with forced enhancer – core-promoter proximity, tethering approaches have also been shown to activate transcription in defined systems [42–44].

Alternatively, the promoter-proximal sequences might, in a cell type-specific manner, regulate the availability of a core-promoter by modulating its DNA accessibility within chromatin (Figure 2C). Transcriptional inactivity due to promoter inaccessibility is also found more generally in *Drosophila* when enhancers skip neighboring genes to activate more distal core-promoters [24], and might also explain the different expression patterns of the divergently transcribed homeobox genes *gooseberry* (*gsb*) and *gooseberry-neuro* (*gsb-n*) [45]: while divergently transcribed homologous genes are often co-expressed, *gsb* and *gsb-n* seem to be differentially active at different embryonic stages, potentially reflecting differential accessibilities of the core-promoter sequences. The regulation of DNA accessibility is also important in other species to regulate core-promoter activity and gene expression (e.g. in worm [46,47]) or of entire gene loci [48–50] and is likely involved in controlling enhancer – core-promoter targeting more generally.

Sequence-encoded Enhancer–core-promoter Specificity

DNA accessibility however does not always explain why neighboring genes are differentially expressed. In *Drosophila*, neighboring genes *out at first (oaf)* and *decapentaplegic (dpp)* are expressed in different patterns at the same stage, despite the *oaf* core-promoter being more proximal to the *dpp* enhancer. Furthermore, if the *oaf* core-promoter is replaced by the *hsp70* core-promoter, *oaf* is activated in the *dpp* expression pattern, arguing that the respective core-promoter sequences are important determinants of enhancer-targeting [51]. In addition, during maternal and early zygotic transcription many genes initiate at different transcription starting sites that can be very closely spaced yet located within AT- versus CG-rich core promoters, suggesting that core-promoter sequences

are involved [52]. This is further supported by the observation that reporter genes under the control of different enhancer – core-promoter combinations can also exhibit distinct expression patterns [53–55], even when integrated at identical genomic positions [56]. In addition, the regulation of several Hox genes including *ftz* by the *caudal* (*cad*) TF seems to depend on specific sequence elements within the core-promoters, particularly the Downstream-Promoter-Element (DPE) [57].

Apart from the DPE, other defined core-promoter motifs or elements also exist and are differentially distributed in core-promoters of genes with different functions. For example, core-promoters of developmentally regulated genes tend to contain TATA box, Initiator (Inr) and DPE motifs, while core-promoters of housekeeping genes contain motifs such as DNA Replicating Elements (DREs), and Ohler Motifs 1, 6, 7 and 8. (reviewed in refs. [58,59]). This sharp dichotomy strongly indicates the involvement of core-promoter sequences in enhancer specificity.

Given the broadness of differential core-promoter element distribution between the developmental and housekeeping gene regulatory programs, the activity of millions of *Drosophila* enhancer candidates towards several housekeeping and developmental core-promoters [60] were recently tested using STARR-seq [61]. This revealed thousands of enhancers with strong preference toward either one of the two core-promoter classes (Figure 3A). In the defined reporter set-up the core-promoters were the only variable: proximity, DNA accessibility, insulators or other DNA elements cannot explain the marked enhancer – core-promoter specificity, suggesting that core-promoter sequences fall into different functional classes, activated by distinct types of enhancers.

Differential Occupancy of *Trans* Factors at Housekeeping versus Developmental Enhancers

The identification of thousands of enhancers with preference towards either one of two core-promoter classes enabled sequence comparison underlying their specificity. As expected, developmental enhancers were enriched for motifs of cell type-specific TFs such as Serpent (Srp), Traffic jam (Tj) and Chorion factor 2 (Cf2) and for dinucleotide repeats, particularly GA repeats that are bound by Trithorax-like (Trl) [62]. In contrast, housekeeping enhancers were enriched for DREs, recognized by the DNA replication-related element factor (DREF). DREs were required and sufficient for housekeeping enhancer activity and allowed the reprogramming of developmental into housekeeping enhancers. Consistently, Trl and DREF were also differentially bound to both types of enhancers. In addition, depletion or specific inhibition of Trl or DREF results in different gene expression responses [63–67], further supporting that the two factors play different roles in gene regulation. The distinctive distribution of TF motifs and the differential binding of the corresponding TFs suggest that the core-promoter-specificity is encoded in enhancer sequences and mediated by *trans*-activating factors [60] (Figure 3B).

Differential Activity of *Trans* Factors at Housekeeping versus Developmental Core-promoters

While sequence motifs and TF occupancy are differentially distributed between housekeeping versus developmental enhancers, these observations do not shed light into how the TFs exert their functions and whether they themselves have intrinsic core-promoter preferences as well. The regulatory activity of different TFs or cofactors on core-promoters can be assessed by directly tethering the respective factors via heterologous DNA-binding-domains (DBDs) to positions upstream of reporter gene core-promoters in *activator bypass* experiments [42–44,68]. Following this logic, 812 *Drosophila* TFs and cofactors were recruited to a housekeeping (hkCP) and a developmental (dCP) core-promoter [69]. Consistent with the differential TF binding to housekeeping and developmental enhancers [60], recruitment of DREF and Trl recapitulated the differential activation of hkCP (by DREF) and dCP (by Trl; Figure 4A).

Interestingly, many additional TFs show differential activities towards the two core-promoters (Figure 4A). For example, Putzig (Pzg) preferentially activates hkCP and is indeed important for housekeeping gene expression [70,71]. On the other hand, TFs that preferentially activated dCP represent factors important during fly development, including the hox TF Abdominal-B (Abd-B), the early zygotic activator Zelda (Zld) and the developmental TFs Cf2 and Pointed (Pnt).

Transcriptional cofactors similarly exhibit core-promoter preferences: dCP is strongly activated by the Mediator subunits MED15 and MED25 as well as the Drosophila CBP/p300 ortholog Nejire (nej), while hkCP is strongly activated by Chromator, Males absent on the first (Mof), TBP-associated factor 4 (Taf4) and Trithorax-related (Trr). The latter type of factors indeed play roles in cell upkeep: for example, Mof is the acetyltransferase component of the Male Specific Lethal (MSL) [72–75] and Non-Specific Lethal (NSL) [76,77] complexes that control dosage compensation on male X chromosome and housekeeping genes transcription, respectively, while Chromator is important in maintaining spindle dynamics during mitosis [78] and regulates chromatin structure [79]. Unsurprisingly, there is much evidence that underscores the importance of cofactor recruitment via sequence-specific TFs during transcriptional regulation in different physiological processes. During dauer formation in worms, DAF-16 TF recruits the SWI/SNF complex to activate longevity and stress resistance target genes [80], while the sterol regulatory element binding protein (SREBP) TF recruits p300 and MED15 during lipid homeostasis [81]. Other examples also exist in different systems [82–84]. Interestingly, cofactors can also provide feedback to DNA binding activity of the TFs [85]. Finally, mutation or overexpression of cofactors deregulates the communication between sequence-specific TF binding to target genes, resulting in human pathologies, for example intellectual disability [86] and colorectal cancers [87]. Thus, the ability of enhancers to trigger transcription from their target core-promoters is highly dependent on channeling of the information at the enhancers to the core-promoters via cofactors. Collectively, the existence of factors that differentially activate the two types of core-promoters suggest that these factors might be the *trans* determinants of enhancer–core-promoter specificity.

Differential Protein Occupancy at Housekeeping versus Developmental Core-promoters

The distinct transcriptional outcomes when tethering the same cofactor to different core-promoters in activator bypass experiments suggests that the protein factors at the two core-promoter types are also different (Figure 4B). Consistently, different core-promoters that are activated by the same TF are affected differentially strongly by the depletion of different cofactors, suggesting that they rely on different *trans* factors [88].

Biochemical studies of proteins bound to core-promoters typically used sequences with the canonical TATA box and Inr motifs. Such studies and the recent elucidation of protein-complex structures have revealed that in an archetypical PIC, TFIID serves as the main recognition factor of the core-promoter [89–91]. TFIID consists of TBP along with 12 to 14 TAF subunits: TBP specifically recognizes the TATA box [92–94], while some TAF subunits recognize other core-promoter elements, for example TAF1 and TAF2 bind to Inr [95], and TAF6 and TAF9 bind to DPE [96] (reviewed in [97]). In addition, TAFs can also relay the communication from the enhancers via direct contact with sequence-specific TFs [98] or by being the targets of cofactors (reviewed in [99]).

However, the PIC is far from uniform. Distinct TFIID complexes exist [100–102] that can bind to TATA box and Inr. Further, some of the components seem to be dispensable, including for example, TBP that appears not to be required for transcription, not even at TATA- and Inr-containing core-promoters [103–105]. Furthermore, “canonical” TAFs are dispensable to accurately position the PIC at a core-promoter that contains a hitherto unknown core-promoter element [106], suggesting for different factor requirements for different core-promoter sequence.

Evidence suggests that housekeeping core-promoters, which typically do not contain TATA box or Inr elements, assemble different complexes: the binding proteins for two of the housekeeping-core promoter motifs are known: Motif 1 Binding Protein (M1BP) recognizes Ohler Motif 1 [107], while DREF recognizes DREs [108]. DREF is a part of a large complex that includes other factors including Pzg, an hkCP-specific cofactor (see above), TATA box binding protein-related factor 2 (TRF2) (ref. [109]), and components of the nucleosome remodeling factor complex (NURF) that catalyzes nucleosome sliding downstream of active housekeeping core-promoters [110].

TRF2 has been shown to bind at non-overlapping positions from TBP in the histone gene cluster [111] and controls the expression of ribosomal genes [109]. In addition, it might also function at DPE-harboring core-promoters [112]. Also, while it was found that TAF4 highly activates the hkCP, it also exhibits preferences for DPE- over TATA-containing core-promoters [113]. Some instances have been known where enhancers [53,56] as well as factors [114–116] show TATA over DPE-specific activation, hinting that DPE- and TATA-containing core promoters might represent different subclasses of developmental core-promoters [58]. Indeed, the TATA-box and DPE motifs rarely occur together in the same core-promoters [117,118].

Transcriptional Activation Mediated via Activating Micro-environments

While it is well established that enhancers and core-promoters are either proximal along the linear DNA or spatially close in 3D [41,119,120], the exact details on how regulatory cues are communicated between enhancers and core-promoters are not entirely clear. Increasing evidence suggests that static and rigid protein-protein interactions between these factors are not involved or at least not required (Figure 5A). For example, the activity of intronic enhancers is not disrupted when Pol II crosses them during the transcription of the host gene [121,122]. Further, the finding that a single enhancer can simultaneously activate transcription of two core-promoters that are 15kb apart in a reporter setup in transgenic *Drosophila* embryos [123] also speaks against static protein-protein contacts. On the other hand, the transcription dynamics of mammalian beta- and gamma-globins on a single allele are more consistent with rapid switching of enhancer – core-promoter contacts, suggesting that such dynamics might depend on the experimental system [42]. These observations, as well as others, are incompatible with a scanning model, in which Pol II is recruited to enhancers and then 'scans' to target core-promoters [124,125], or a model in which the Polymerase is 'handed over' from enhancers to core-promoters (recently reviewed in [126,127]).

Such observations are rather compatible with an activating microenvironment around the enhancer, in which enhancer-bound TFs recruit cofactors, thereby increasing their local concentration and ability to activate nearby core-promoters (Figure 5B; see refs. [128,129] for a more detailed review). As different activating cofactors possess enzymatic activities, for example to post-transcriptionally modify other proteins, these activities and post-translational modifications (PTMs) might be involved in transcriptional regulation. Indeed, the activities of many TFs, general TFs of the PIC, Pol II, and some cofactors are modulated by post-transcriptional modifications. The communication from enhancer to core-promoter thus might conceivably be transmitted via post-translational modification of different factors, precluding the requirement of static interactions of the factors. For example, Pol II is acetylated by P300/CBP at certain growth factor responsive genes and this PTM is required for Pol II activity at these genes [130]. Similarly, P300/CBP acetylates hematopoietic TFs that leads to the recruitment of BRD4, promoting the expression of leukemia maintenance genes [131,132]. Post-translational modification of TFs can also modulate the TFs' binding sites and target genes, as for example shown for Mef2 in *Drosophila* [133]. PTMs are also important in regulating specific steps of transcription, for example the release of paused Pol II, and it is conceivable that certain enhancers also regulate this step [134]. These examples highlight the specific activation of some genes via transmitting of information via post-transcriptional modification.

Concluding Remarks and Future

The past years have witnessed enormous progress in our understanding of transcriptional regulation, the organization of animal regulatory genomes, and enhancer – core-promoter communication: insulators restrict enhancer activities and delineate the genome into regulatory domains, long-range enhancer – promoter contacts enable distal regulation, and the cell-type-specific availability of core-promoters can be regulated by DNA accessibility

within chromatin. Furthermore, it is increasingly clear that enhancer – core-promoter specificities can be determined by the sequences of both elements: differential motif distribution allows distinct sets of factors to be recruited at enhancer and core-promoters such that biochemical compatibilities between the factors determine core-promoter targeting and effective regulatory communication.

Sequence-mediated Core–promoter-enhancer Specificity in Other Transcription Programs

As known core-promoter elements correlate with biological functions [58,118,135] and diverse TAFs and their paralogues exist that relate to specific transcription programs (reviewed in [136,137]), it is possible that more transcription programs are separated at the level of enhancer – core-promoter specificity. For example, during *Drosophila* spermatogenesis, an alternate TFIID complex that consists of testis-specific TAFs (tTAFs) is required for transcription of spermatid differentiation genes, but dispensable for meiotic cell cycle genes [138,139]. The regulatory information comes from sequence-specific DNA binding of tMAC complex, which relays the information through MED22 to achieve gene selectivity of tTAFs [140]. It will be exciting to learn about additional transcriptional programs, e.g. in germ cells.

Sequence-encoded enhancer and core-promoter function

Genomic regulatory regions have often been annotated according to their location with respect to genes, including ‘promoter’ regions as regions upstream of gene transcription sites and ‘enhancers’ as TSS-distal. Our ability to measure transcription (initiation) with increasing sensitivity has revealed that both enhancer and promoter regions can initiate transcription and frequently contain (degenerate) core-promoter sequence elements [141–143]. Transcription initiation within enhancers has for example been used to predict enhancers [141,144–148]. Furthermore, functional assays demonstrated that *Drosophila* promoters can partly act as enhancers to activate transcription from a distal target core-promoter [60]. Consistently, human TSS-proximal sequences that can recruit cofactors display transcription initiating (“promoter”) activity when tested without an enhancer, as well as enhancer activity [149]. Typically however, core-promoter activities have been measured in the presence of activating enhancers [55,150,151]. These observations suggest that future definitions might better be based on functional assays that specifically assess enhancer and core-promoter function separately. As enhancers are typically assessed by their ability to activate transcription from a given core-promoter compared to negative controls (reviewed in [6,152]), we propose to assess core-promoter functionality by their ability to convert the activity of a given enhancer into transcription initiation events, i.e. by the core promoters’ enhancer-responsiveness given by its induced versus basal activity. It will be interesting to learn which sequences within a large animal genome possess high enhancer-responsiveness and what the determining sequence properties will be.

Rethinking enhancer–core-promoter communication: activating micro-environments and biochemical compatibilities

We are most excited about the prospects of learning how enhancer-bound TFs and the cofactors they recruit mediate regulatory communication with core-promoters. It is clear that the proteins involved do not form rigid or stable complexes, but rather a dynamic and

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flexible micro-environment that can activate more than one core-promoter simultaneously [123] and the function of which is resilient to structural perturbation by transcription through the enhancer [121,122]. Which combinations of TFs and cofactors are able to activate transcription, how biochemical compatibility is implemented, and which PTMs on TFs, cofactors, histones, or PIC components are involved are exciting open questions for future studies (See Outstanding Questions Box). Perhaps in the future such knowledge could be used for therapeutic purposes, for example to highly precisely activate or repress gene transcription using TALE or Cas9-derived regulators [153,154], entirely synthetic proteins [155,156] or small molecule mimetics [157] or inhibitors [3,158–160].

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Trends

- The specificity of enhancers to core-promoters is encoded in the sequence.
- Motifs within enhancer and core-promoter sequences recruit *trans* acting factors that mediate regulatory enhancer – core-promoter communication and specificity.
- The communication is mediated by a high local concentration of cofactors that interact dynamically with and possibly post-transcriptionally modify each other and the Polymerase.

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Outstanding Questions

- What are the defining sequence features of a core-promoter that allow high enhancer-responsiveness?
- How many functionally distinct types of core-promoters exist? Are there additional types of enhancer–core-promoter specificities employed for other transcription programs (e.g. in germ cells)?
- How are enhancer – core-promoter specificities implemented molecularly?
- How is the information from enhancer is communicated to the core-promoter? Do different enhancers use different modes of communication?
- Which combinations of TFs activate transcription? How is combinatorial control achieved molecularly?
- How many different types of TFs exist, how many are obligate combinatorial?
- Which TFs recruit which of the cofactors and what are the respective protein domains or interaction surfaces?
- Which PTMs are involved, what are the key PTM target proteins? Are there consensus signals?

Glossary

Cofactors: regulatory protein factors that are typically unable to bind to DNA themselves and are recruited to enhancers by TFs. Cofactors can have enzymatic functions, for example catalyze post-translational modifications of proteins, and mediate the regulatory function of the enhancers.

Core-promoter: short sequence around the TSS that can direct the recruitment of Polymerase II and transcription initiation. Core-promoters typically have low basal activities in the absence of enhancers and are also called *minimal promoters*.

Enhancer: sequence of several tens to hundred bps that boosts transcription from a target core-promoter in a cell-type-specific manner and independent of the enhancer's relative orientation and distance.

Insulators: DNA elements that can block regulatory enhancer – core-promoter communication, typically by the recruitment of insulator proteins such as CTCF, and thus demarcate the range of enhancer activity and define TAD borders.

Pre-Initiation Complex (PIC): an assembly of proteins, including Polymerase II and general transcription factors, that first nucleates at core-promoter before transcription initiation, and makes Polymerase II transcription competent.

Promoter: sequence up to several kbs upstream of TSSs that can autonomously drive transcription. This functionality and the fact that some promoters can activate transcription from a distal core-promoter in reporter assays is in line with promoters consisting of a core-promoter and a proximal or overlapping enhancer.

Promoter-proximal tethering elements: sequences proximal to the core-promoter that enable or facilitate its interaction with enhancers.

Promoter-targeting sequences: sequences proximal to enhancers that enable or facilitate their interactions with core-promoters.

STARR-seq (self-transcribing active regulatory region sequencing): enhancer-activity assay in which candidate DNA fragments are positioned in the 3'UTR of a reporter gene such that active enhancers transcribe themselves and the sequences' activities can be measured by their abundance among cellular RNAs [61]. The coupling of candidate sequences to enhancer activity in *cis*, such that each enhancer serves as its own barcode, allows the parallel assessment of millions of DNA fragments from arbitrary sources and enables genome-wide functional enhancer screens.

Transcription factors (TFs): proteins that bind DNA in enhancers through their DNA binding domains and activate or repress transcription, usually via the recruitment of cofactors.

TF recognition sequences or binding sites (vs. motifs): short DNA sequences that bind TFs. Consensus sequences that summarize a TF's binding preference are called TF motifs.

Topologically associating domains (TADs): Large genomic regions, typically several kilo or megabase pairs long, within which frequent chromatin contacts occur, as measured by chromosome-conformation-capture (3C) and variant techniques. As discussed in the main text, the importance of TADs for transcriptional regulation is increasingly being recognized.

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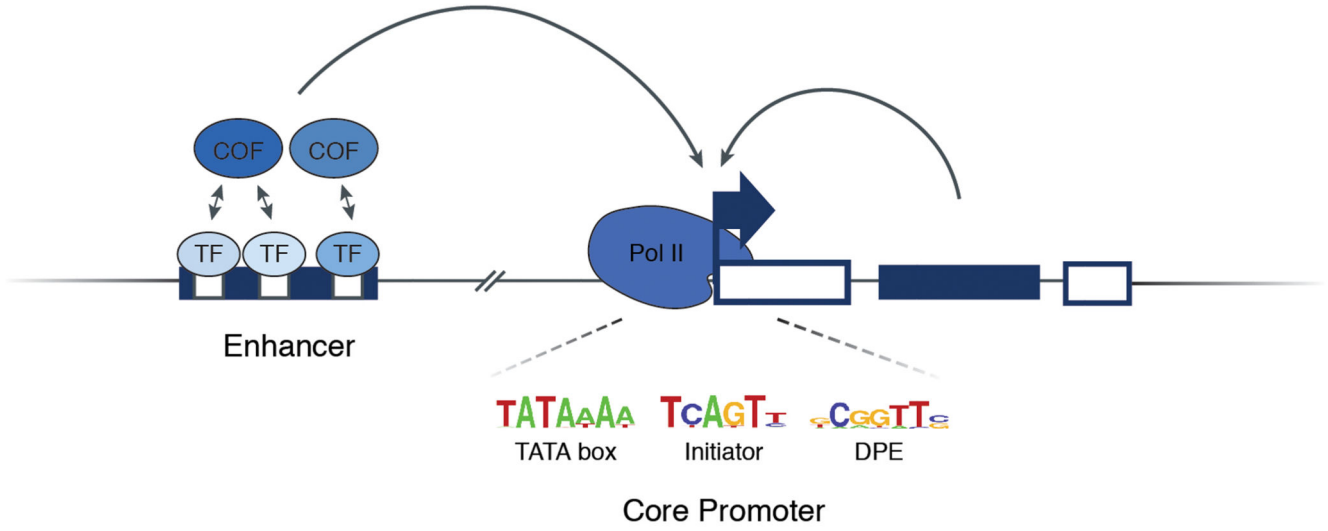

Figure 1. Enhancers and core-promoters, two major classes of *cis*-regulatory elements.

An enhancer contains binding sites for sequence-specific transcription factors (TFs). These in turn recruit cofactors (COFs) that typically mediate the regulatory communication between the core promoter and the enhancer, i.e. relay the enhancer's regulatory cues to the enhancer's target core promoters. Core-promoters encompass short sequences of ~100bp surrounding the transcription start site, where Polymerase II (Pol II) assembles and initiates transcription. Core-promoters typically contain characteristic core-promoter elements or motifs, for example TATA box, Initiator or Downstream Promoter Element (DPE).

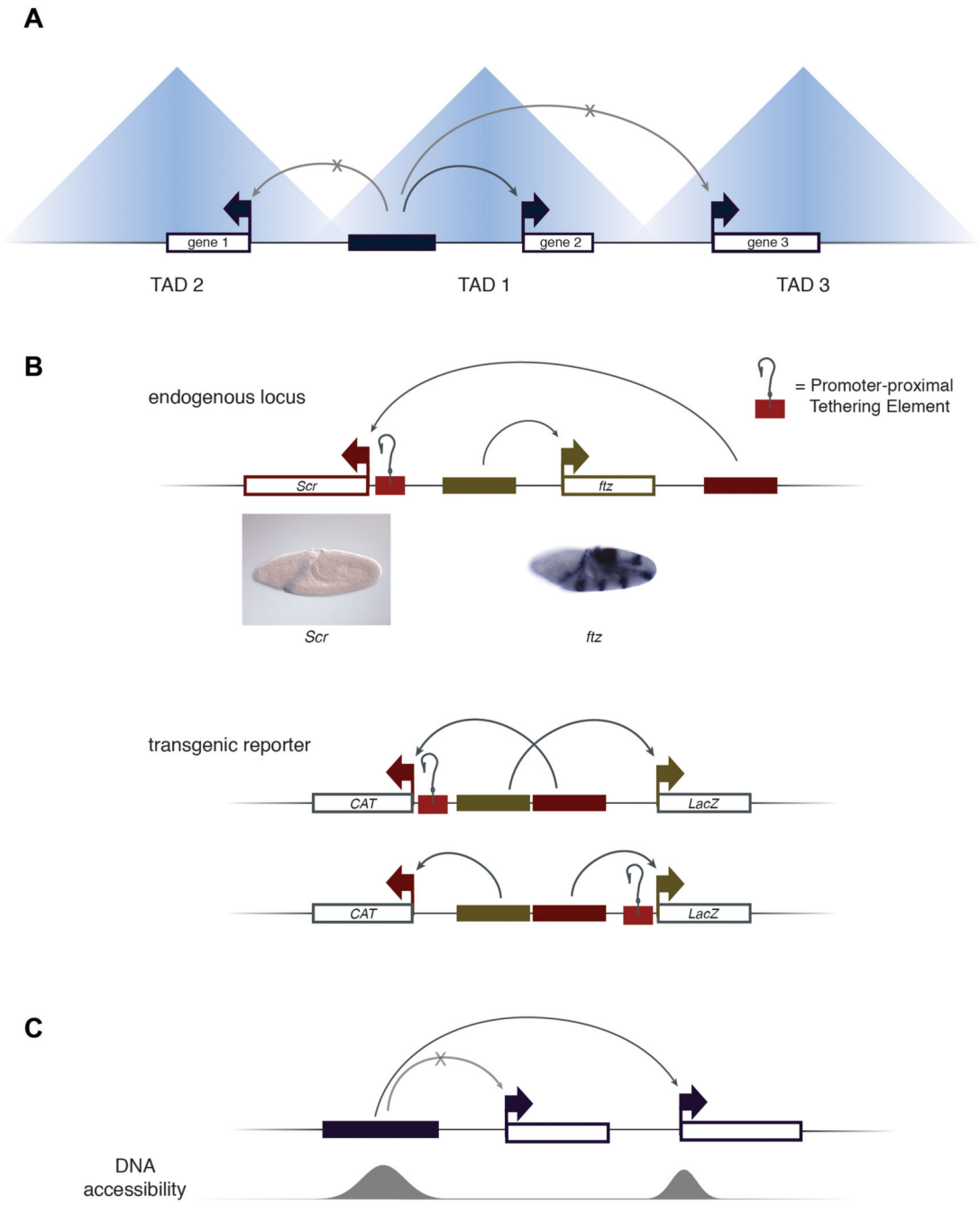

Figure 2. Topologically associating domains (TADs), promoter-proximal tethering elements (PTEs), and DNA accessibility.

(A) Enhancer function is typically restricted to activate core-promoters within the same TADs [12,31], the boundaries of which are enriched in insulator protein binding. **(B)** PTEs are sequences proximal to core-promoters that promote the preferential interaction between enhancers and core-promoters [37–39]. For example, the PTE of the *Sex combs reduced (Scr)* core-promoter enables its activation by the distally-located *Scr* enhancer, skipping the intervening *fushi tarazu (ftz)*. In transgenic reporter, relocation of the PTE to a proximal

position of the *ftz* core-promoter results in activation of the latter by the *Scr* enhancer. *In situ* staining of embryo images are from Berkeley Drosophila Genome Project [161]. **(C)** Inaccessible DNA precludes a *cis*-regulatory element from participating in potential interaction. For example, *Drosophila* enhancers skip the proximal and inaccessible core-promoters, to activate more distal and accessible core-promoters [24].

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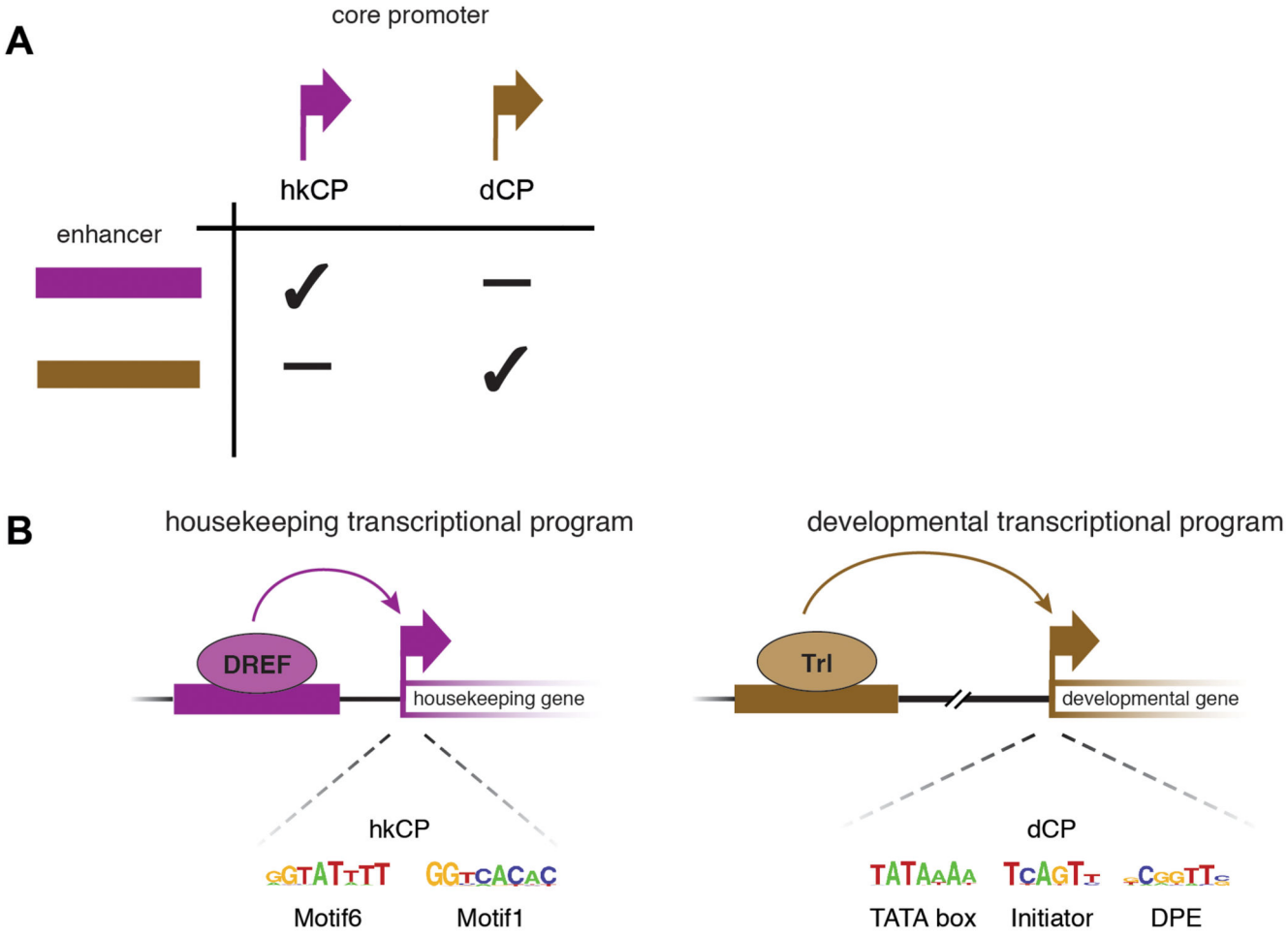

Figure 3. Sequence-mediated enhancer–core-promoter specificity.

(A) housekeeping core promoters (hkCP; purple) show preferences towards housekeeping over developmental enhancers. The reverse is true for developmental core promoters (dCP; ochre; ref [60]). **(B)** housekeeping enhancers bind DREF and activate transcription from hkCP, which typically contain Ohler Motifs 1 and 6 core-promoter elements (indicated by motif logos). Developmental enhancers meanwhile bind Trl and activate transcription from dCPs that contain different core-promoter elements, such as TATA box, Initiator and DPE. Compared to housekeeping enhancers which are TSS-proximal or even overlapping, developmental enhancers are found at various positions, including within introns or very distal.

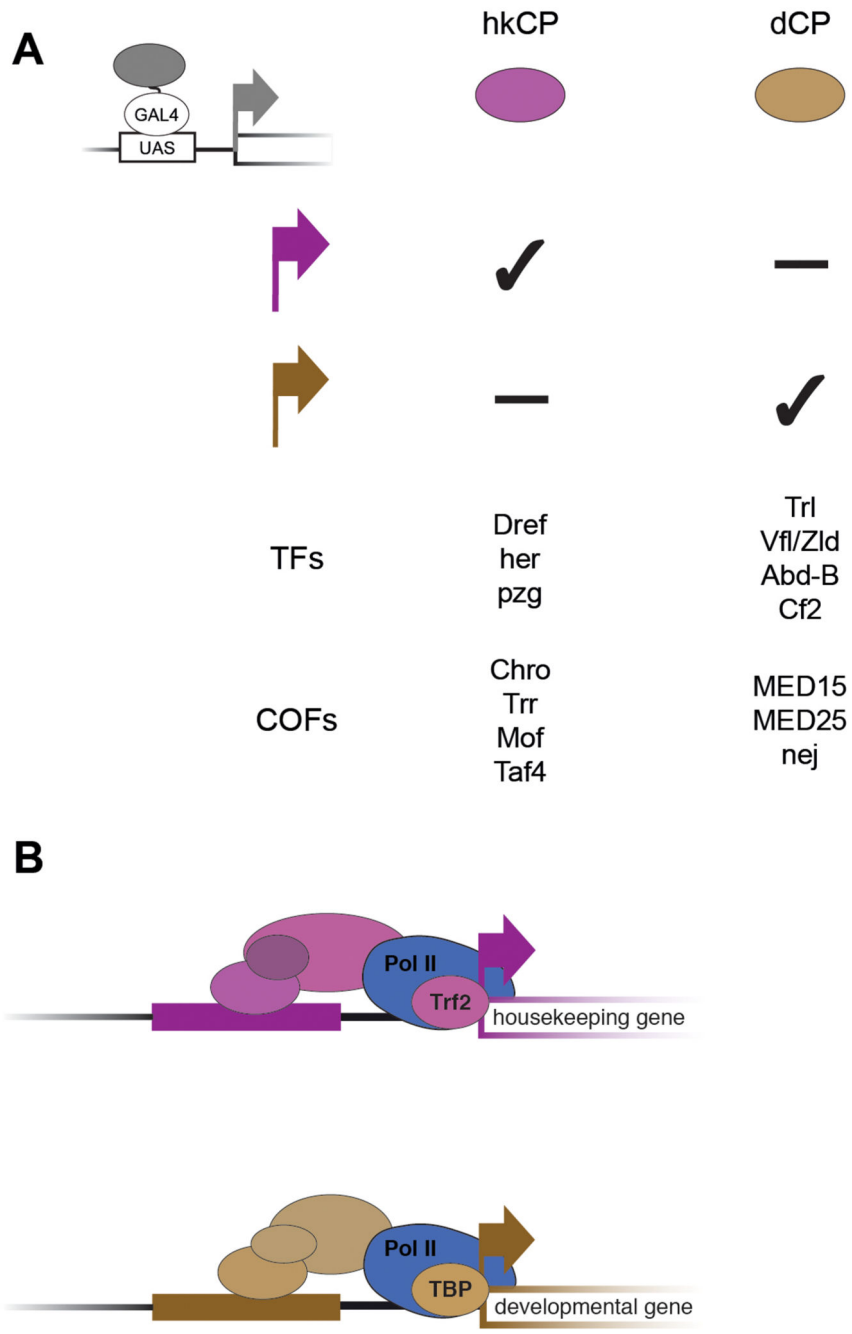

Figure 4. ***Trans*** **factors mediate sequence-mediated enhancer–core-promoter specificity.**
(A) Activator bypass experiments show that different transcription factors (TFs) and cofactors (COFs) can differentially active hkCP over dCP. Activation is indicated by a check mark and examples of TFs and cofactors that function accordingly are shown below. **(B)** hkCP and dCP recruit TRF2- and TBP-containing complexes, respectively. The members of these complexes also potentially differ.

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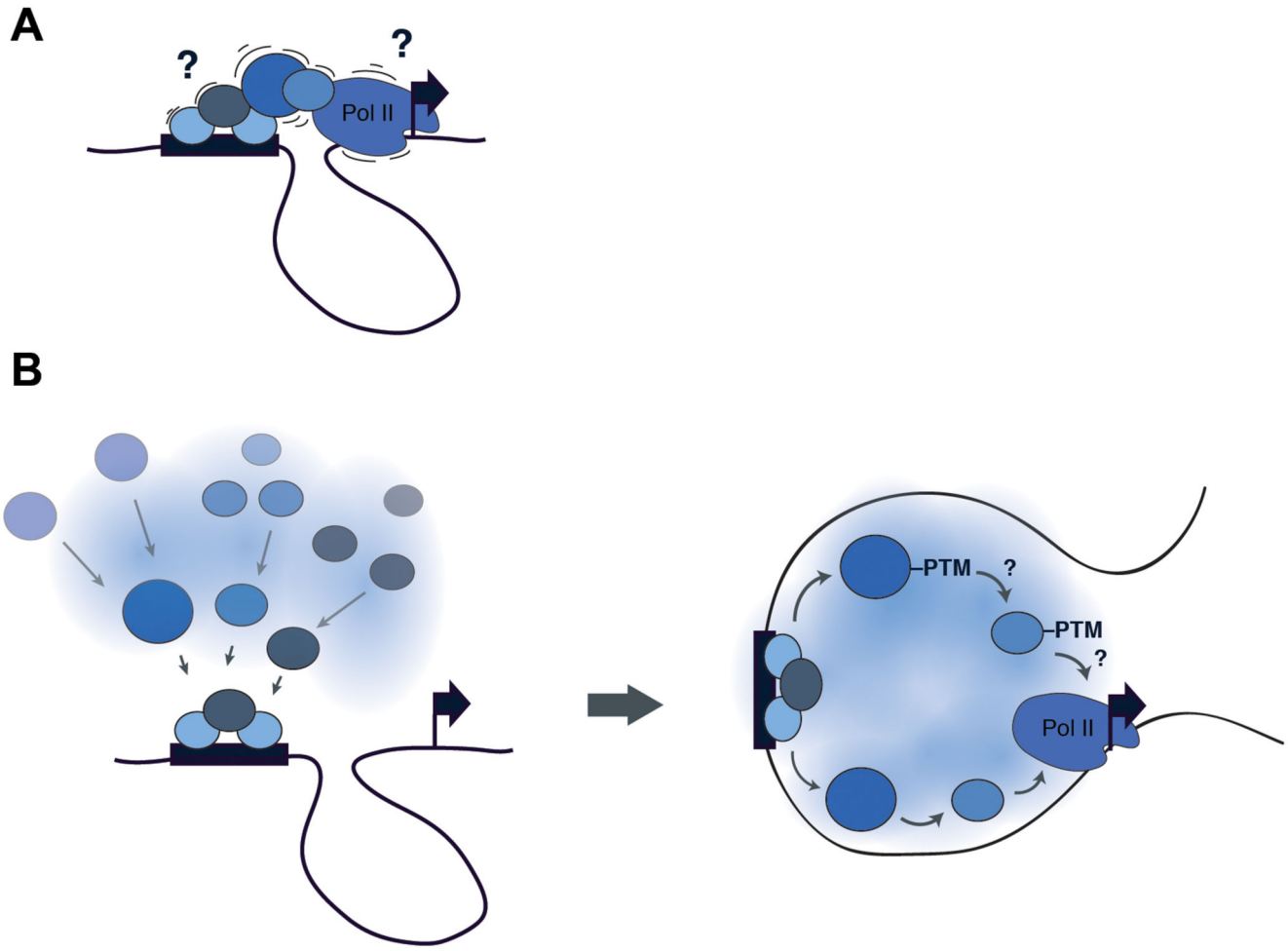

Figure 5. Enhancers activate core-promoters in a microenvironment.

(A) Static model of transcription regulation in which defined protein complexes formed by static protein-protein interactions at enhancers exert their function on core promoters. This model is incompatible with some observations such as the simultaneous activation of two core-promoters by a single enhancer [123]. **(B)** Transcription regulation might alternatively occur via an activating microenvironment, in which enhancers and core-promoters recruit *trans* factors to create a high concentration of regulatory proteins that dynamically interact with each other, and enable regulatory communication through post-transcription modifications (PTMs, top) or dynamic protein-protein-interactions and recruitment (bottom).