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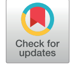
Molecular models of bidirectional promoter regulation



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Abstract

Approximately 11% of human genes are transcribed by a bidirectional promoter (BDP), defined as two genes with <1 kb between their transcription start sites. Despite their evolutionary conservation and enrichment for housekeeping genes and oncogenes, the regulatory role of BDPs remains unclear. BDPs have been suggested to facilitate gene coregulation and/or decrease expression noise. This review discusses these potential regulatory functions through the context of six prospective underlying mechanistic models: a single nucleosome free region, shared transcription factor/regulator binding, cooperative negative supercoiling, bimodal histone marks, joint activation by enhancer(s), and RNA-mediated recruitment of regulators. These molecular mechanisms may act independently and/or cooperatively to facilitate the coregulation and/or decreased expression noise predicted of BDPs.

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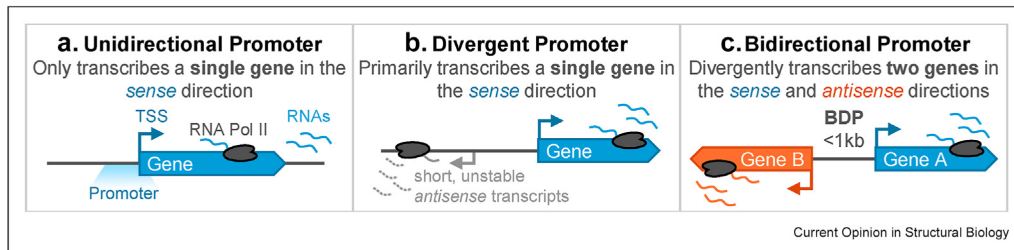
Introduction

Precise gene regulation is essential for proper development, maintenance of homeostasis, and response to extracellular stimuli. Mammalian gene regulation begins with transcription, which initiates at a gene's promoter. For this review, a gene's promoter is defined as the region upstream and encompassing a gene's transcription start site, where regulatory proteins and transcriptional machinery bind to direct transcription initiation. Productive transcription requires (1) promoter activation, wherein a promoter enters a transcriptionally permissive chromatin state, followed by (2) transcription initiation, (3) elongation, and (4) termination, resulting in a full-length mature transcript [1–3]. First, transcription factors (TFs) and cofactors bind their cognate sites at enhancers and/or promoters, and recruit the transcriptional machinery, consisting of RNA polymerase II (Pol II) and general transcription factors (GTFs). Pol II and GTFs then form the pre-initiation complex (PIC) at the gene's transcription start site (TSS). Following initiation, Pol II begins transcription elongation through the gene body followed by termination and polyadenylation. Mammalian transcription as well as additional regulatory steps not discussed in this review including promoter-proximal pausing, 5′ capping, and splicing have been reviewed in further detail by Roeder [3], Core and Adelman [4], Ramanathan et al. [5] and Wilkinson et al. [6].

Transcriptional initiation at promoters is often described as unidirectional, wherein unidirectional promoters transcribe a single gene in a single direction (Figure 1a). However, studies using nascent transcript sequencing estimate that 48.9% [7] to >75% [8,9] of human promoters are divergently transcribed. These estimates vary based on sequencing and analysis methods, as discussed in Duttke et al. (2015) [10]. Divergent promoters transcribe a single gene in the sense direction as well as a short, rapidly degraded upstream RNA (sometimes referred to as uaRNAs, CUTS, PROMPTS, etc.) in the antisense direction (Figure 1b) [7–9,11–16]. Transcription at divergent promoters initiates from separate core promoters and PICs [8,9,11,12,17,18]. However, distinct from both unidirectional and divergent promoters are bidirectional promoters. A bidirectional promoter (BDP) is defined as two divergently transcribed genes with less than 1000 base pairs between their transcription start sites (Figure 1c) [19,20]. Like divergent promoters, transcription at bidirectional promoters initiates from separate core promoters and PICs [8,9,11,12,17,18,21]. However, in contrast to divergent promoters, BDPs specifically transcribe two full-length, stable transcripts. These transcripts include protein-coding genes and (non)functional non-coding transcripts, such as lncRNAs and miRNAs [19,20]. For this review, BDP gene pairs are referred to as *Forward (Watson) Gene/Reverse (Crick) gene.*

Figure 1



Types of mammalian promoters. a. A unidirectional promoter transcribes a single gene in the sense direction. **b.** A divergent promoter primarily transcribes a single gene in the sense direction, but also divergently initiates antisense transcription. Sense and antisense transcripts at divergent promoters are initiated from separate core promoters; however, productive RNA elongation resulting in mature RNAs only occurs in the sense direction. Antisense transcripts terminate early (<350bp) and are rapidly degraded. **c.** A bidirectional promoter divergently transcribes two genes with <1000bp between their TSSs. The sense and antisense genes are initiated from separate core promoters. The distinguishing factor between divergent and bidirectional promoters is that BDPs transcribe two full-length genes; these genes can include protein-coding transcripts as well as stable, non-coding transcripts.

Approximately 11% of human genes are transcribed by a bidirectional promoter [19,20,22,23]. These genes are enriched for important functionalities: 35% of housekeeping genes [19], 33.3% of DNA repair genes [20], and 23% of oncogenes [19,24] are under a BDP. Furthermore, the bidirectional promoter architecture tends to be conserved at these genes, suggesting BDPs serve an important regulatory function [20,25]. However, the regulatory function(s) of BDPs are currently poorly understood, largely due to limited study of BDPs. Despite our limited knowledge, prior studies of bidirectional and divergent promoters have suggested multiple potential regulatory function(s), including coregulation and lower noise, and mechanistic models for BDPs.

Models of BDP regulatory function

Coregulation of BDP genes

BDPs have been hypothesized to coregulate their two genes. For this review, coregulation is defined as two genes covarying (increasing or decreasing concordantly) over time within a single cell as well as between cells in a genetically identical population [26]. Gene coregulation is important for maintaining the stoichiometry of proteins that form complexes or function together in pathways. Coregulation can be achieved through several mechanisms, including transcriptional coregulation via shared transcription factors [27–29], post-transcriptional coregulation via miRNAs [30], and post-translational coregulation via coordinated protein degradation [31]. In this review we focus on BDP-mediated coregulation.

Given our understanding of promoters and transcriptional regulation, it is possible that bidirectional promoters may broadly exhibit coregulation at the transcriptional level; this coregulation may be attenuated at the RNA and/or protein levels, as observed in Kustatscher et al. (2017). This aligns with studies showing that genes sharing a BDP are typically not functionally related [19,20,22,32] and that while many BDP genes are evolutionarily conserved under the bidirectional promoter architecture, their gene partner is generally not [20]. Additionally, 11% of BDP gene pairs exhibit anticorrelated expression [32]. In total, the evidence suggests that coregulation may not be a universal function of BDPs [20]; however, coregulation may still be a key function for some BDP gene pairs, such as the *HTB2/HTA2* bidirectional promoter in yeast, which requires precise protein stoichiometry for histone functionality [33–35].

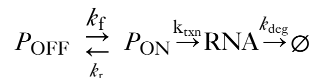
Transcriptional noise

In addition to coregulation, BDPs have also been hypothesized to decrease transcriptional noise [25]. For this review, transcriptional noise is defined as the variation in a single gene's RNA levels between cells in a genetically identical population (variance/mean^2) [36]. Transcriptional noise generally propagates, resulting in expression noise at the protein level [36–38]. Expression noise is an important regulatory feature. In yeast, certain gene functionalities generally exhibit higher (e.g. stress response genes) or lower (e.g. housekeeping) expression noise [22,25,34,39–42]. Given their enrichment for low-noise genes [19,20,25], it is plausible that bidirectional promoters may decrease expression noise. Indeed, by analyzing single-cell protein abundance in yeast, Wang et al. (2011) found that BDP genes exhibited significantly lower expression noise than non-BDP genes [25]. This finding held true when considering all genes as well as only non-essential genes [25].

A major source of expression noise is stochastic, "bursty" transcription due to promoters alternating between active (ON) and inactive (OFF) states [1,37,43–45]. During each burst (ON state), multiple RNAs may be transcribed. As such, some cells may have many RNAs while others have none, resulting in variation (noise) in both RNA and protein levels. Transcriptional bursting is

likely universal for all eukaryotes [46] and has been observed in yeast [47], drosophila [48], mice [49], and humans [50].

The biophysical and regulatory properties of BDPs may modulate transcriptional noise through transcriptional bursting. To illustrate this, consider a 2-state promoter model, where the promoter alternates between an ON and OFF state [37,51,52] as shown below:



$$P_{\mathrm{OFF}} \underset{k_r}{\overset{k_f}{\rightleftarrows}} P_{\mathrm{ON}} \xrightarrow{k_{txn}} \mathrm{RNA} \xrightarrow{k_{deg}} \varnothing$$

This model has four rate parameters: forward rate (k_f for $P_{OFF} \rightarrow P_{ON}$), reverse rate ($k_r$ for $P_{ON} \rightarrow P_{OFF}$), the rate of transcription during the ON state (k_{txn}), and the rate of RNA degradation (k_{deg}). Here we hold the transcription (k_{txn}) and degradation (k_{deg}) rates constant and consider only changes in promoter transition rates (k_f, k_r). An increase in only the forward (k_f) rate results in a decreased OFF state duration (time between bursts), increased transcriptional output, and decreased transcriptional noise. A decrease in only the reverse rate (k_r) results in an increased ON state duration, increased transcriptional output, and decreased transcriptional noise. In the limit of an extremely long ON state duration and/or extremely short OFF state duration, the promoter approaches the low-noise Poisson limit of constitutive expression [53]. Thus, BDPs could reduce noise in at least two ways: (1) increasing the ON-duration without affecting other parameters; (2) decreasing the OFF-duration without affecting other parameters. Both result in reduced noise and increased average transcriptional output. This aligns with the observed lower expression noise [25] and high expression levels of BDP genes [7,14,19,54].

Mechanistic models of BDP regulation

Here we discuss potential mechanistic models for bidirectional promoter regulation that may contribute to the transcriptional coregulation and/or decreased expression noise predicted of BDPs. These models include: a single shared nucleosome free region (Figure 2a), shared regulator binding (Figure 2b), cooperative negative supercoiling (Figure 2c), bimodal histone modifications (Figure 2d), coregulation by enhancers (Figure 2e), and RNA-mediated regulation (Figure 2f). These non-exclusive models may contribute individually or combinatorially to the regulation of transcription at divergent and/or bidirectional promoters.

Nucleosome free region

Transcriptionally active regions of the genome are associated with a nucleosome free region (NFR). Promoters are typically associated with an NFR of approximately 100−300 bp length, flanked by a strongly positioned +1 nucleosome and a weaker positioned −1 nucleosome [55]. The NFR exposes the naked DNA sequence, facilitating TF and regulator binding as well as PIC formation [8,17,56−59]. In unidirectional promoters, the PIC forms directly upstream of the +1 nucleosome. At divergent and bidirectional promoters, divergent transcripts initiate from two separate PICs at the strongly positioned +1 and −1 nucleosomes, separated by a single shared NFR (Figure 2a) [8,9,11,12,17,18,21]. Nucleosome free regions at divergent and bidirectional promoters are typically 100-300bp in length, but may span upwards of 1000bp [8]. This aligns with the TSS distances of bidirectional promoters, which are enriched for short TSS distances (between ∼100 and 300bp [8,13,19,32]) but by definition can have a TSS distance of up to 1000bp [32].

Bidirectional promoter genes sharing a single NFR could contribute to coregulation. Nucleosome repositioning and eviction could create a transcriptionally permissive state for both genes, resulting in coordinated transcription activation. Downstream regulator binding, PIC formation, and histone modifications could then modulate each gene's transcription initiation, resulting in BDPs with varying levels of coregulation.

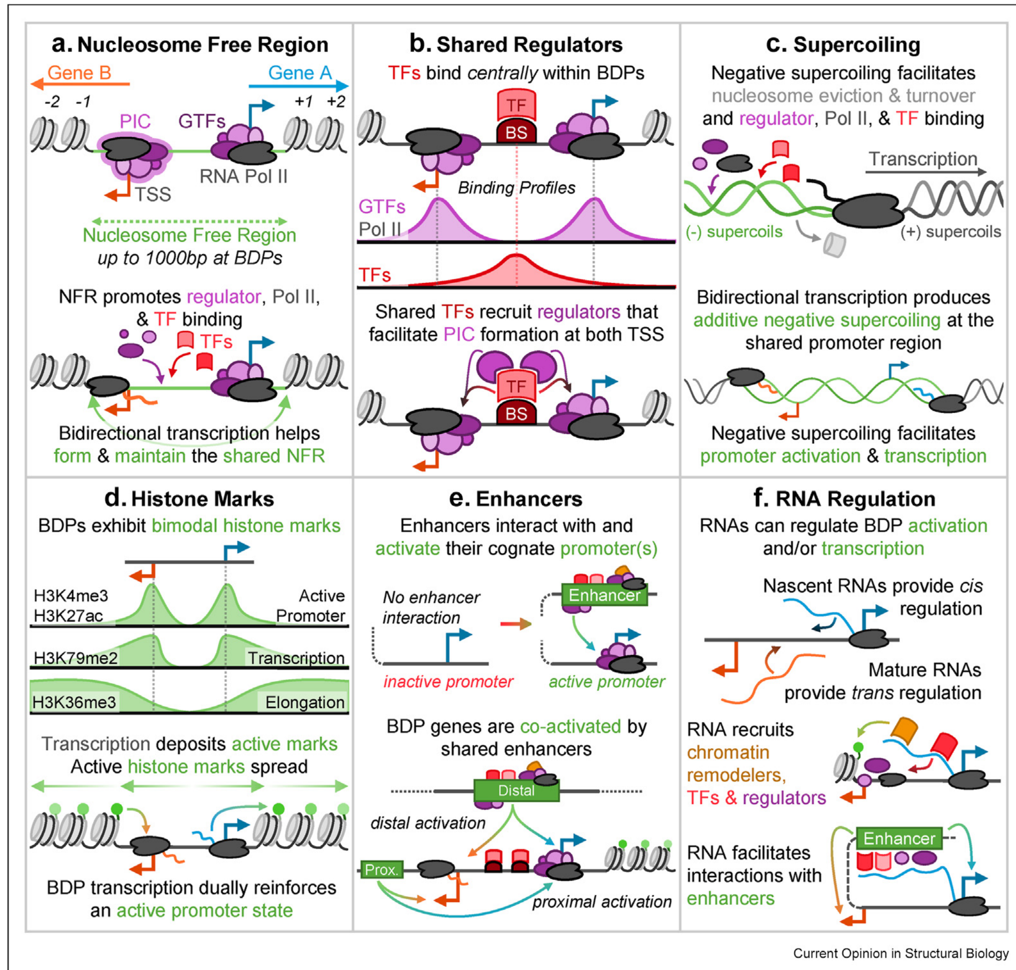
Divergent transcription could also reinforce the NFR, resulting in more consistent expression and decreased noise. Studies have suggested that transcription along with the recruitment of chromatin remodelers may contribute to maintaining a transcriptionally permissive NFR through regulating nucleosome composition, positioning, and turnover [56,57,60−63] (Figure 2a). Divergent transcription at divergent and bidirectional promoters could dually reinforce this feedback loop [13,34], such that the transcription in the sense or antisense direction can help promote the formation and maintenance of the shared NFR, collectively increasing the promoter's ON duration and decreasing transcriptional noise.

Shared regulator binding

A gene's promoter is characterized by the binding of regulatory proteins (such as TFs, GTFs, and Mediator) and RNA Pol II and its cofactors. While divergent and bidirectional promoters have separate core promoters and PICs, the binding of regulatory proteins may be shared. Given evidence that separate genes regulated by the same TF are coregulated [27−30], it is possible that BDP genes sharing a TF binding site will exhibit some degree of coregulation. This effect would be protein-dependent, as some regulators enforce promoter directionality [7,17,62,64,65].

Supporting this hypothesis, Core et al. (2014) found that at divergent and bidirectional promoters, the majority (73/84) of TFs bind centrally between the TSSs, suggesting these TFs may regulate both BDP genes

Figure 2



Mechanistic models for bidirectional promoter regulation. a. Bidirectional promoters are marked by a single, shared NFR spanning the distance between the two genes' TSSs. This NFR facilitates protein binding, PIC formation, and transcription, which in turn may maintain the NFR. **b.** Many transcription factors bind to their binding site (BS) centrally within BDPs, suggesting they may regulate both BDP genes. This is seen at the *GAL80/SUT-719* BDP in yeast, where Gal4 facilitates PIC formation at both TSS. **c.** Divergent transcription at BDPs produces additive negative supercoiling, which may facilitate an active promoter state through promoter melting, nucleosome modulation, and protein binding. **d.** BDPs exhibit bimodal distributions for active promoter (H3K4me3, H3K27ac), transcription (H3K79me2), and elongation (H3K36me3) histone marks. Transcriptionally linked deposition and spread of these histone marks may reinforce an active promoter state. **e.** BDPs may be coregulated by distal and proximal regulatory elements including enhancers, as observed at the *NIPBL/NIPBL-AS1* BDP in humans, where the expression of both genes decreases upon the deletion of a distal enhancer. **f.** Nascent and mature transcripts produced by BDPs may regulate the promoter through the recruitment of regulatory proteins and/or facilitating interactions with distal regulatory elements.

(Figure 2b). In contrast, GTFs bind proximally to the forward and reverse TSS [11,66], aligning with each TSS having its own PIC. These results agree with CryoEM studies showing that the Gal4-VP16 activator binds centrally at the *GAL80/SUT719* BDP in yeast [21]. The Gal4-VP16 dimer recruits a Mediator-PIC dimer to the BDP, which is then delivered to each gene's core promoter via DNA bending and GTFs [21].

Coregulation of BDPs by a shared TF in mammals has also been observed. Expression of the *SERPINI1/PDCD10* BDP gene pair is coregulated by c-MYC [67]. The *PRR11/SKA2* and *MRPS12/SARS2* BDP gene pairs are both characterized by shared NF–Y binding sites [68–70]. However, both gene pairs have multiple NF–Y binding sites which exhibit different regulatory effects.

In addition to the direct effects of TF binding, divergent transcription may result in a reservoir of transcriptional machinery [7,12,34]: the unbinding of regulators and disassembly of PIC components at the +1 and −1 transcription start sites could result in a high local concentration of TFs and transcriptional

machinery, allowing for rapid reinitiation of transcription at divergent and bidirectional promoters [34,71]. This may decrease gene expression noise caused by stochastic turnover of TF, GTF, and Pol II [58,72]. This also aligns with observed higher TF binding and overall expression levels at BDPs compared to divergent or unidirectional promoters [7,11,14,54,66].

Supercoiling

As RNA Pol II transcribes, it produces positive DNA supercoils ahead of itself and negative DNA supercoils in its wake (Figure 2c) [73]. Divergent transcription at bidirectional promoters produces additive negative supercoiling at the shared promoter region [13,47,74–76]. Negative supercoiling has been proposed to facilitate nucleosome eviction/turnover, promoter melting, TF and Pol II binding, and PIC formation (Figure 2c) [73,74,77–81]; thus, cooperative negative supercoiling at the promoter region could promote transcriptional activation. Indeed, models of supercoiling at divergently oriented promoters predict that cooperative negative supercoiling could result in gene coregulation, as observed at the *her7* and *her1* locus in zebrafish [75,82,83]. This model predicts that this supercoiling may increase transcription factor and RNA Pol II binding, increase promoter ON duration, and decrease OFF duration, potentially decreasing transcriptional noise [75]. Additive negative supercoiling is likely more pronounced at BDPs, which transcribe full length genes, than at divergent promoters, as negative supercoiling produced during transcription increases with transcript length. The interplay between supercoiling, nucleosome occupancy, and TF binding could further amplify the positive regulatory impact of supercoiling at BDPs, creating a cooperative system for maintaining transcriptional activity.

However, the accumulation of excessive negative supercoiling at the promoter region has also been shown to inhibit BDP transcription and coregulation, as observed at the *GAL1/GAL10* bidirectional promoter in yeast [47]. This suggests that bidirectional promoters may require a certain level of supercoiling to achieve efficient transcription and/or coregulation.

Histone marks

Active and inactive promoter states are associated with distinct histone post-translational modifications (histone marks) [84]. While certain histone marks correlate with active transcription, it remains unclear whether these marks occur upstream or downstream of transcription [84]. Specifically, it has been proposed that (1) histone marks may direct transcription initiation and elongation, as these processes are distinguished by distinct histone marks [55,84,85]; and/or (2) histone marks are deposited via transcription, as evidenced by histone readers/writers interacting directly with RNA Pol II [84–86].

Multiple studies have shown that BDPs exhibit distinct histone profiles. At active promoters, transcription initiation is associated with H3K4me3 and H3K27ac while transcription and elongation are associated with H3K79me2 and H3K36me3 [84]. These marks are differentially enriched at unidirectional, divergent, and bidirectional promoters. Unidirectional promoters only contain H3K4me3, H3K27ac, H3K79me2, and H3K36me3 in the sense direction [7,9,11,12,16]. Divergent promoters exhibit bimodal enrichment for H3K4me3 and H3K27ac but only contain elongation marks in the sense direction [7,9,11,12,16]. Bidirectional promoters exhibit bimodal enrichment for H3K4me3, H3K27ac, H3K79me2, and H3K36me3 (Figure 2d) [7,9,11,12,16,87,88], aligning with BDPs transcribing two full-length genes in both directions. Whether this bimodality is due to co-deposition and/or spread of histone marks between the two genes or if it is merely a consequence of divergent transcription is currently unknown. Regardless, bimodal active histone marks suggest some degree of co-activation within BDP gene pairs. Furthermore, BDPs exhibit strong and wider H3K4me3 and H3K27ac signals [14]. This suggests a strong activation state, possibly resulting in longer ON states and decreased noise. However, as our knowledge of histone mark functionality is limited [84], the causality and effects of BDP histone mark profiles is difficult to dissect.

Enhancers

Enhancers are a key regulator of gene expression [89]. Upon interacting with their cognate promoter(s), enhancers deposit chromatin remodelers, TFs, and/or transcriptional machinery, facilitating promoter activation and transcription initiation (Figure 2e) [90,91]. Studies have suggested that a single enhancer can regulate multiple promoters [48,92–94]. Therefore, it is possible that a single enhancer could coregulate two genes sharing a BDP. Indeed, at the *NIPBL/NIPBL-AS1* BDP in human cells, deletion of a 130 kb distal enhancer resulted in a concordant ∼40% decrease in the transcription of both genes [95]. Similar results were observed at the *DEIN/HAND2* and *NBR2/BRCA1* BDPs, where multiple proximal enhancers/silencers were found that regulate both genes [96–98]. These shared enhancer interactions and resultant activation likely contribute to the observed coexpression of these BDPs. However, it is possible that enhancer coregulation of BDPs is not universal and may be context (BDP, enhancer, cell-type) specific.

RNA regulation

There is increasing evidence that nascent and mature transcripts may perform local regulatory functions, including the recruitment of TFs and chromatin modifiers via RNA-binding domains [99–101], and distal regulatory functions, including facilitating interactions with distal enhancers (Figure 2f) [102,103]. Thus, at

some BDPs, particularly those containing protein-coding/lncRNA gene pairs, the transcription of Gene A may recruit regulatory factors to further direct and tune the transcription of Gene B. Indeed, RNA regulation of BDPs was observed at the *BISPR*/*BST2* BDP gene pair. siRNA knockdown of the *BISPR* lncRNA resulted in decreased *BST2* expression, while transgenic over-expression of *BISPR* resulted in increased *BST2* expression, suggesting the *BISPR* gene product enacts *trans* regulation on *BST2* [104].

RNA-mediated regulation could also tune coregulation and/or transcriptional noise at BDPs. Positive regulation, where Gene A's RNA recruits regulators that facilitate the activation of Gene B, could result in coregulation as well as decreased expression noise by decreasing Gene B's OFF duration via facilitating its activation and/or increasing Gene B's ON duration via maintaining its active promoter state. Negative regulation, where Gene A's RNA recruits regulators that repress Gene B, could result in negative coregulation and anticorrelation between Gene A and B. However, RNA regulation is less likely to occur at divergent promoters, as antisense RNAs are short and unstable, which may limit their ability to perform regulatory functions.

A combinatorial model for BDP regulation

In total, these six cooperative, non-independent molecular mechanisms are consistent with a BDP regulation model in which: (1) BDP Gene A is activated which then facilitates the activation of BDP gene B; and/or (2) both BDP genes are simultaneously activated. Both scenarios result in gene coregulation, while scenario (1) also results in decreased noise due to decreased OFF duration. Divergent transcription then maintains a joint transcriptionally active state at the BDP, increasing ON duration and decreasing expression noise. This regulatory model may not be universal, particularly at BDPs which exhibit antagonistic expression; however, these molecular mechanisms are still broadly compatible with positive, negative, and independent BDP RNA regulation.

Antagonism in BDPs

While prior studies generally observe BDP coregulation [19,20,25,32,39], some studies have observed antagonistic expression at BDPs. Trinklein et al. (2004) found that 11% of BDPs exhibit negative expression correlation between their two genes, while 10% transcribe in only one direction [32]; additionally, at 9/10 BDPs, the deletion of Gene A's TSS resulted in increased expression of Gene B [32]. Similarly, Zuin et al. (2017) found that dCas9 transcriptional blocking of Gene A increased the transcription of Gene B and vice versa for the *NIPBL*/*NIPBL-AS1* BDP [95]. However, *NIPBL* and *NIPBL-AS1* exhibit coregulation by their shared enhancer. This suggests nuanced regulation at this BDP. Understanding mechanistically how different BDPs exhibit cooperative, antagonistic, or independent regulation is an important direction for future studies.

Technical considerations of studying BDPs

Elucidating the regulatory role of BDPs and exploring the contributions of these mechanistic models has been difficult, partly due to technical limitations. Measuring covariance is complicated, as different techniques (fluorescent/luminescent reporter assay [25,32,35,69,87,96,98,105,106], microarray [20,32,107], qPCR [88,95,102,104], bulk and single-cell RNA-seq-based methods [8,14,15,19,22,62,86,88,108,109], RNA-FISH [35,65], etc.) measure different types of covariance (transcription, RNA, protein) [30,110–112] in different contexts (monoclonal population, different lines of the same cancer, different tissue types, etc.). Multiple studies have suggested that BDPs exhibit some degree of coregulation, more than predicted solely by sharing a genomic context [19,20,25,32,39]; however, other studies argue that BDP coregulation is not universal and may not be functional. Yang et al. (2007) found that BDP expression correlation decreases as more tissue types are considered [107]. Kustatscher et al. (2017) observe mRNA covariance at 31/167 (19%) BDPs in lymphoblastoid cell lines, but of these BDPs only 3/31 also covary at the protein level [22]. Other studies have observed independent or anticorrelated expression at some BDPs [32,95]. As such, it remains unclear whether BDPs are generally coregulated or if this coregulation is functional. Further studies of bidirectional promoters with improved techniques will allow us to better understand the generality, mechanisms, and function of BDP coregulation.

Specifically, current high-throughput techniques are poorly equipped to measure transcriptional coregulation, transcriptional bursting, and transcriptional noise. Recently developed single cell GRO-seq and equivalent techniques are promising, as they allow for single-cell measurement of coregulation via Pol II localization on nascent RNA [27]. From these measurements, transcriptional bursting can be extrapolated more accurately than other RNA-seq based techniques. However, scGRO-seq suffers from technical noise and requires optimization to achieve sufficient measurement efficiency [27,113]. Static RNA imaging approaches, such as single-molecule RNA-FISH [35], provide a snapshot of nascent transcript levels across a cell population and can measure noise. Live-cell nascent RNA imaging [47,48,50] can directly measure nascent transcription over time and can thus measure transcriptional coregulation, bursting dynamics, and transcriptional noise. However, these imaging approaches tend to be low-throughput and experimentally intensive. A combination of imaging techniques with improved single-cell nascent RNA sequencing methods may provide a clearer picture regarding BDP coregulation.

Conclusion

Despite their evolutionary conservation and regulatory importance, BDPs remain poorly understood. Prior studies have provided preliminary insights into their regulatory functions and mechanisms. However, further studies of individual and broad BDP dynamics through the scope of the molecular models outlined in this review using techniques that can directly measure nascent transcription will allow us to elucidate the regulatory role of BDPs and improve our understanding of transcriptional regulation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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