Transcribe this way: Rap1 confers promoter directionality by repressing divergent transcription

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ABSTRACT
In eukaryotes, divergent transcription is a major source of noncoding RNAs. Recent studies have uncovered that in yeast, the transcription factor Rap1 restricts transcription in the divergent direction and thereby controls promoter directionality. Here, we summarize these findings, propose regulatory principles, and discuss the implications for eukaryotic gene regulation.

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Introduction
Eukaryotic gene promoters are inherently bidirectional [1]. This process, known as divergent or bidirectional transcription, generates upstream transcripts in the opposing direction to the coding gene from a distinct core promoter (Figure 1) [2]. The divergent transcripts produced are typically unstable and are a major source of noncoding RNAs [3,4].

The functions of divergent noncoding transcription, and the RNAs generated, are not well understood. Many divergent noncoding transcripts, but not all, are likely the non-functional products of “noisy” transcription [5,6]. Some roles for divergent transcription have been proposed. For example, divergent transcription may facilitate new gene formation. Non-functional enhancer RNAs and divergent transcripts can be co-opted for biological functions by evolutionary pressures [7]. Transcription at gene promoters is inherently bidirectional, but evolutionary forces shift bidirectional output towards unidirectional coding gene transcription over time [1]. Divergent transcripts themselves can also regulate gene expression directly in cis or in trans [8–11].

Mis-expression of divergent transcripts may compromise cellular fitness. In organisms like Saccharomyces cerevisiae, the distance between genes is relatively short. As a consequence, divergent transcription can overlap with neighboring genes and cause transcriptional interference [12]. If neighboring genes are oriented in tandem, for example, divergent transcripts can overlap with upstream genes as antisense long noncoding RNAs. Inappropriate noncoding transcription can also generate R-loops in vivo, which compromises genomic stability [13,14]. In addition, aberrant divergent transcription is wasteful and energetically costly for cells [15]. To complete the transcription cycle, many macromolecular machines are produced, assembled, and recruited to DNA. Therefore, cells must have robust mechanisms to limit the inappropriate expression of divergent transcripts.

Expression of divergent RNAs is controlled at multiple steps during gene expression. Pathways involved in chromatin structure, RNA termination, and RNA degradation play significant roles in limiting the accumulation of divergent transcripts [6]. For example, histone modifications like H3K56 (histone H3 lysine 56) acetylation and variants like H2A.Z regulate divergent transcription by modulating nucleosome assembly and remodeling [16,17]. In addition, control of TATA-binding protein activity can also limit divergent and pervasive transcription, as can RNA polymerase speed [18–20]. Divergent transcription of non-coding RNAs is also controlled by gene looping...
and chromatin conformation [21]. Finally, transcription termination and RNA turnover limit accumulation of aberrant transcripts. The Nrd1-Nab3-Sen1 (NNS) and premature polyadenylation signal (PAS) pathways in yeast and mammalian cells, respectively, terminate and degrade divergent promoter transcripts [22, 23]. The Integrator complex is also likely involved in termination of divergent transcription [14]. Exosome and nonsense-mediated decay pathways degrade cryptic and divergent RNAs, limiting their expression [20, 24, 25]. Together, these pathways limit the presence of pervasive divergent RNAs.

**Rap1, a transcriptional activator that represses divergent transcription**

We recently identified that the transcription factor Rap1 confers promoter directionality by specifically repressing initiation of divergent noncoding transcription [26]. Rap1 has multiple functions in yeast. This pioneer transcription factor is essential for telomere and hidden mating type silencing, and activates highly expressed ribosomal protein (RP) and glycolytic genes [27–29]. In parallel with a study by Challal et al., we showed that rapid depletion of Rap1 leads to widespread induction of divergent transcripts very close to sites where Rap1 is bound [26, 30]. We validated this proximity-dependent effect of Rap1 binding through mutagenesis at representative RP gene and divergent promoters. Without Rap1, divergent transcripts can disrupt regulatory circuits controlling cell fate decisions or interfere with gene expression at mRNA and protein levels. These examples illustrate the importance of controlling promoter directionality at very active promoters. Thus, Rap1 represses divergent transcription at hundreds of highly expressed genes throughout the yeast genome.

How does Rap1 repress divergent transcription? We investigated whether chromatin regulators or co-repressors are required for Rap1-mediated transcriptional repression. We found that Rap1 and other chromatin regulators repress discrete divergent or cryptic antisense promoters at distinct genomic locations, and are not redundant. Rap1 most likely represses divergent transcription directly, because its cofactors and interacting partners do not inhibit divergent transcription from Rap1-regulated promoters.

In contrast, Rap1 does not repress divergent transcription from gene promoters as a transcriptional “roadblock”. Rap1 and a related transcription factor, Reb1, can terminate elongating RNA polymerase and prevent read-through transcription from interfering with downstream gene expression [31–33]. However, Rap1 binding sites at gene promoters are extremely close to divergent transcription start sites (TSSs) – within 0–50 base pairs (bp) – which would interfere with transcription initiation instead. In addition, there is no potential roadblock posed by Rap1 for most divergent transcripts, as most of their TSSs are upstream (not downstream) of promoter Rap1 binding sites. We tested this model experimentally by repositioning the Rap1 binding sites in the RPL43B promoter 400 bp downstream of the

![Diagram](image-url)

**Figure 1.** Schematic diagram of a bidirectional gene promoter.

A bidirectional gene promoter comprises separate core promoters (blue boxes) for the coding messenger RNA (mRNA) and divergent RNA, arranged in opposite orientations. These core promoters are typically located at the edges of a nucleosome-depleted region, flanked by the +1 and −1 nucleosomes (gray circles). The relative amount of transcription from each core promoter dictates the overall “directionality” of the gene promoter. The scale bar illustrates that the output of eukaryotic gene promoters ranges widely: some promoters are more unidirectional, whereas others display more bidirectional transcription.
divergent transcript IRT2 TSS in a potential “roadblock” position, and found that the divergent transcript was not effectively repressed. In addition, proximal Rap1 binding sites located upstream of an independent divergent TSS were already sufficient to repress divergent transcription [26]. Thus, Rap1 limits divergent transcript expression by regulating transcription initiation, not elongation. We propose that a stable physical association between Rap1 and its target sequences at promoters can achieve repression of divergent transcription. Therefore, Rap1 may specifically block or reduce the association of transcriptional activators and general transcription machinery to the divergent core promoter.

The steric hindrance model is attractive for several reasons. First, Rap1 is ideally positioned to restrict initiation of divergent transcription, typically within 50 bp of its binding sites. Rap1 binds at the 5’ (upstream) edge of the promoter nucleosome-depleted region (NDR), where divergent transcription initiates. Steric hindrance of the divergent core promoter is spatially limited and does not interfere with gene transcription in the coding direction. Rap1 binding sites in vivo are several hundred base pairs upstream of coding direction TSSs. If the Rap1 binding site(s) were repositioned in close proximity to the coding TSS, away from the divergent TSS, we would expect divergent transcription to increase while coding direction transcription would decrease. Second, the physical association of Rap1 with its target motif confers effective transcriptional repression independent of Rap1 motif orientation (Figure 2). Finally, Rap1 maintains a stable association with DNA during different cellular states, in contrast to other RP gene coactivators that dissociate from the promoter after stress [34]. To maintain repression of divergent noncoding transcription, Rap1 must stably bind its target motifs at promoters. Limiting the recruitment of basal transcription machinery

![Figure 2](image-url)

**Figure 2.** Requirements for steric hindrance of divergent transcription initiation. (a) Transcription factor binding site (red boxes) must be within ~50 bp of divergent transcription start site (TSS) for effective repression of transcription. Repression at proximal binding sites does not depend on specific Rap1 motif orientation. (b) Rap1 binding at distal sites, upstream or downstream of the divergent TSS, does not effectively limit the expression of divergent transcripts.
could be an efficient way to limit divergent transcription at highly expressed genes. The intrinsic features of Rap1-regulated genes may justify the use of this specialized mechanism to regulate divergent transcripts. Highly transcribed genes tend to have wider NDRs, and promoters activated by Rap1 are among the most active in yeast. Therefore, it is not surprising that Rap1-regulated promoters contain a wide NDR approximately 200–400 bp in length, compared to 150 bp for the average yeast promoter [35,36]. The wide NDR generated by Rap1, together with coactivators and chromatin remodelers, facilitates proper coding gene activation [27,28,37]. Without stringent control by Rap1 and its cofactors, open chromatin could allow inappropriate recruitment of RNA polymerase II. Subsequently, aberrant transcription could proceed in both directions from the distinct coding and divergent core promoters that occupy the outer borders of NDRs [38]. Rap1 depletion also shifts TSS usage upstream in the sense direction, which compromises coding gene expression in many cases [26,30]. We propose that Rap1 reduces the association of initiation factors, basal transcription machinery, and ATP-dependent chromatin remodelers at sequences surrounding the Rap1 binding site, to stimulate productive transcription in the protein-coding direction only. In other words, Rap1 is positioned to repress initiation of divergent transcription, while concurrently facilitating orderly recruitment of cofactors to drive transcription in the coding gene direction.

Transcriptional repression using steric hindrance

The ability to repress transcription using steric hindrance is not unique to Rap1. Gene regulation through steric hindrance is widespread through all three domains of life, viruses, and synthetic repression systems (not a comprehensive list).

| Table 1. Examples of transcriptional repression by steric hindrance in different organisms. |
| --- |
| Factor | Species or Origin | Reference |
| **Bacteria** |  |  |
| Trp repressor | E. coli | Kumamoto et al., 1987[59] |
| LexA repressor | E. coli | Little et al., 1981[59] |
| Lac repressor | E. coli | Brent and Ptashne, 1981[40] |
| **Archaea** |  |  |
| MDR1 repressor | A. fulgidus | Bell et al., 1999[60] |
| LrpA repressor | P. furiosus | Brinkman et al., 2000[61] |
| Phr heat shock response regulator | P. furiosus | Vierke et al., 2003[62] |
| Eukaryotes |  |  |
| AP2 | H. sapiens | Getman et al., 1995[63] |
| Glucocorticoid receptor (GR) | B. taurus | Sakai et al., 1988[64] |
| Rap1, likely Reb1 & Abf1 | S. cerevisiae | Wu et al., 2018[26] |
| **Viruses** |  |  |
| cl and Cro | Lambda (λ) bacteriophage | Challal et al., 2018[30] |
| T antigen | SV40 | Meyer et al., 1975[65] |
| LBP-1 (host factor) | HIV-1 | Johnson et al., 1978[66] |
| **Synthetic systems** |  |  |
| dCas9 (catalytic inactivated Cas9 mutant) | From S. pyogenes | Qi et al., 2013[43] |
| TALEs | From Xanthomonas sp. | Gilbert et al., 2013[42] |

Some examples of transcriptional repression through steric hindrance are listed, from different sources including all three domains of life, viruses, and synthetic repression systems (not a comprehensive list).
S. cerevisiae, Rap1 has been co-opted to drive most RP gene expression by transcription factor motif substitution [46]. Other transcription factors such as Abf1, Reb1, Tbf1, and Cbf1 also share structural homology with the Rap1 DNA binding domain, possess "pioneer" nucleosome displacement activity, and drive RP gene expression in other fungal species [47,48]. These transcription factors may also fulfill the requirements for steric hindrance of divergent transcription; this hypothesis requires experimental validation. In higher eukaryotes, certain sequence-specific transcription factors may perform analogous roles in the regulation of divergent transcription. Recent work has assessed the contribution of chromatin states and core promoter sequence towards promoter directionality in metazoans [49]. A number of pioneer transcription factors that open DNA asymmetrically were also identified, belonging to the Klf/Sp, NFYA, Creb/ATF, and Zfp161 families [50]. These transcription factors are present at the edges of NDRs at promoters and enhancers, where divergent core promoters are also located, and thus are ideally positioned to repress divergent transcription.

Comparing closely or distantly related species can highlight key regulatory principles controlling the expression of divergent transcripts [1]. Some eukaryotes, like Drosophila melanogaster, were thought to have little to no divergent transcription [51]. However, technical advances in the detection of nascent transcription uncovered widespread expression of divergent transcripts, which are unstable in many cases [52,53]. In Arabidopsis seedlings, GRO-seq (global run-on sequencing) and NET-seq (native elongating transcript sequencing) approaches have revealed low amounts of detectable divergent transcription at RNA polymerase II promoters [54,55]. Coincidentally, plant genomes harbor hundreds of transcription factors with Myb-like DNA-binding domains similar to Rap1 in yeast, while vertebrate genomes only contain a handful of Myb-like proteins. Myb is a conserved DNA binding protein found in retroviral oncogenes, and organisms ranging from sea urchins to humans [56,57]. Typically, these transcription factors control transcriptional responses to proliferation, differentiation, and environmental stresses [58]. It would be interesting to examine whether the expansion of Myb-related transcription factor gene families and the Myb domain specifically repress divergent transcription in plants and other organisms.

Conclusion

In conclusion, stable binding of sequence-specific transcription factors to cis-regulatory elements can limit divergent noncoding transcription and thus control promoter directionality. Recent work highlights one way in which the information encoded in cis-regulatory elements can be interpreted by trans-acting regulatory proteins like transcription factors to produce a transcriptional output. It is possible that other sequence-specific transcription factors and DNA binding proteins limit cryptic transcription near regulatory elements, as shown for Rap1.

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References

[1] Jin Y, Eser U, Struhl K, et al. The ground state and evolution of promoter region directionality. Cell. 2017;170:889–98 e10.
[2] Andersson R, Chen Y, Core L, et al. Human gene promoters are intrinsically bidirectional. Mol Cell. 2015;60:346–347.

[3] Seila AC, Calabrese JM, Levine SS, et al. Divergent transcription from active promoters. Science. 2008;322:1849–1851.

[4] Xu Z, Wei W, Gagneur J, et al. Bidirectional promoters generate pervasive transcription in yeast. Nature. 2009;457:1033–1037.

[5] Struhl K. Transcriptional noise and the fidelity of initiation by RNA polymerase II. Nat Struct Mol Biol. 2007;14:103–105.

[6] Jensen TH, Jacquier A, Libri D. Dealing with pervasive transcription. Mol Cell. 2013;52:473–484.

[7] Wu X, Sharp PA. Divergent transcription: a driving force for new gene origination? Cell. 2013;155:990–996.

[8] Frank S, Ahuja G, Bartsch D, et al. ylincT defines a class of divergently transcribed IncRNAs and safeguards the T-mediated mesodermal commitment of human PSCs. Cell Stem Cell. 2019;24:318–27 e8.

[9] Grote P, Wittler L, Hendrix D, et al. The tissue-specific lncRNA Fendr is an essential regulator of heart and body wall development in the mouse. Dev Cell. 2013;24:206–214.

[10] Du Mee DJM, Ivanov M, Parker JP, et al. Efficient termination of nuclear lncRNA transcription promotes mitochondrial genome maintenance. Elife. 2018;7:e31989.

[11] Bumgarner SL, Dowell RD, Grisafi P, et al. Toggle involving cis-interfering noncoding RNAs controls variegated gene expression in yeast. Proc Natl Acad Sci U S A. 2009;106:18321–18326.

[12] Ard R, Allshire RC, Marquardt S. Emerging properties and functional consequences of noncoding transcription. Genetics. 2017;207:357–367.

[13] Hamperl S, Bocek MJ, Saldivar JC, et al. Transcription-replication conflict orientation modulates R-loop levels and activates distinct DNA damage responses. Cell. 2017;170:774–86 e19.

[14] Nojima T, Tellier M, Foxwell J, et al. Deregulated expression of mammalian lncRNA through loss of SPT6 induces R-loop formation, replication stress, and cellular senescence. Mol Cell. 2018;72:970–84 e7.

[15] Lynch M, Marinov GK. The bioenergetic costs of a gene. Proc Natl Acad Sci U S A. 2015;112:15690–15695.

[16] Marquardt S, Escalante-Chong R, Pho N, et al. A chromatin-based mechanism for limiting divergent noncoding transcription. Cell. 2014;158:462.

[17] Rege M, Subramanian V, Zhu C, et al. Chromatin dynamics and the RNA exosome function in concert to regulate transcriptional homeostasis. Cell Rep. 2015;13:1610–1622.

[18] Xue Y, Pradhan SK, Sun F, et al. Mot1, Ino80C, and NC2 function coordinately to regulate pervasive transcription in yeast and mammals. Mol Cell. 2017;67:594–607 e4.

[19] Fong N, Saldi T, Sheridan RM, et al. II dynamics modulate co-transcriptional chromatin modification, CTD phosphorylation, and transcriptional direction. Mol Cell. 2017;66:546–57 e3.

[20] Neil H, Malabat C, d’Aubenton-Caraf Y, et al. Widespread bidirectional promoters are the major source of cryptic transcripts in yeast. Nature. 2009;457:1038–1042.

[21] Tan-Wong SM, Zaug JB, Camblong J, et al. Gene loops enhance transcriptional directionality. Science. 2012;338:671–675.

[22] Almada AE, Wu X, Kriz AJ, et al. Promoter directionality is controlled by U1 snRNA and polyadenylation signals. Nature. 2013;499:360–363.

[23] Ntini E, Jarvelin AI, Bornholdt J, et al. Polyadenylation site-induced decay of upstream transcripts enforces promoter directionality. Nat Struct Mol Biol. 2013;20:923–928.

[24] van Dijk EL, Chen CL, d’Aubenton-Caraf Y, et al. XUTs are a class of Xrn1-sensitive antisense regulatory non-coding RNA in yeast. Nature. 2011;475:114–117.

[25] Malabat C, Feuerbach F, Ma L, et al. Quality control of transcription start site selection by nonsense-mediated mRNA decay. Elife. 2015;4:e06722.

[26] Wu ACK, Patel H, Chia M, et al. Repression of divergent noncoding transcription by a sequence-specific transcription factor. Mol Cell. 2018;72:942–54 e7.

[27] Azad GK, Tomar RS. The multifunctional transcription factor Rap1: a regulator of yeast physiology. Front Biosci (Landmark Ed). 2016;21:918–930.

[28] Hu H, Li X. Transcriptional regulation in eukaryotic ribosomal protein genes. Genomics. 2007;90:421–423.

[29] Shi T, Bunker RD, Mattarocci S, et al. Rif1 and Rif2 shape telomere function and architecture through multivalent Rap1 interactions. Cell. 2013;153:1340–1353.

[30] Challal D, Barucco M, Kubik S, et al. General regulatory factors control the fidelity of transcription by restricting non-coding and ectopic initiation. Mol Cell. 2018;72:955–69 e7.

[31] Colin J, Candelli T, Porrua O, et al. Roadblock termination by Reb1p restricts cryptic and readthrough transcription. Mol Cell. 2014;56:667–680.

[32] Candelli T, Challal D, Briand JB, et al. High-resolution transcription maps reveal the widespread impact of roadblock termination in yeast. EMBO J. 2018;37:e97490.

[33] Yarrington RM, Richardson SM, Lisa Huang CR, et al. Novel transcript truncating function of Rap1p revealed by synthetic codon-optimized Ty1 retrotransposon. Genetics. 2012;190:523–535.

[34] Reja R, Vinayachandran V, Ghosh S, et al. Molecular mechanisms of ribosomal protein gene coregulation. Genes Dev. 2015;29:1942–1954.

[35] Bai L, Morozov AV. Gene regulation by nucleosome positioning. Trends Genet. 2010;26:476–483.

[36] Weiner A, Hughes A, Yassour M, et al. High-resolution nucleosome mapping reveals transcription-dependent promoter packaging. Genome Res. 2010;20:90–100.

[37] Kubik S, O’Duibhir E, de Jonge WJ, et al. Sequence-directed action of RSC remodeler and general
regulatory factors modulates +1 nucleosome position to facilitate transcription. Mol Cell. 2018;71:89–102 e5.

[38] Albert I, Mavrich TN, Tomsho LP, et al. Translational and rotational settings of H2A.Z nucleosomes across the Saccharomyces cerevisiae genome. Nature. 2007;446:572–576.

[39] Little JW, Mount DW, Yanisch-Perron CR. Purified lexA protein is a repressor of the recA and lexA genes. Proc Natl Acad Sci USA. 1981;78:4199–4203.

[40] Brent R, Ptashne M. Mechanism of action of the lexA gene product. Proc Natl Acad Sci U S A. 1981;78:4204–4208.

[41] Sellitti MA, Pavco PA, Steege DA. lac repressor blocks in vivo transcription of lac control region DNA. Proc Natl Acad Sci U S A. 1987;84:3199–3203.

[42] Gilbert LA, Larson MH, Morsut L, et al. CRISPR-mediated modular RNA-guided regulation of transcription in eukaryotes. Cell. 2013;154:442–451.

[43] Qi LS, Larson MH, Gilbert LA, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell. 2013;152:1173–1183.

[44] Clauß K, Popp AP, Schulze L, et al. DNA residence time is a regulatory factor of transcription repression. Nucleic Acids Res. 2017;45:11121–11130.

[45] Li Y, Jiang Y, Chen H, et al. Modular construction of mammalian gene circuits using TALE transcriptional repressors. Nat Chem Biol. 2015;11:207–213.

[46] Hugues H, Lavoie H, Sellam A, et al. Transcription factor substitution during the evolution of fungal ribosome regulation. Mol Cell. 2008;29:552–562.

[47] Bosio MC, Fermi B, Dieci G. Transcriptional control of yeast ribosome biogenesis: A multifaceted role for general regulatory factors. Transcription. 2017;8:254–260.

[48] Yan C, Chen H, Bai L. Systematic study of nucleosome-displacing factors in budding yeast. Mol Cell. 2018;71:294–305 e4.

[49] Ibrahim MM, Karabacak A, Glahs A, et al. Determinants of promoter and enhancer transcription directionality in metazoans. Nat Commun. 2018;9:4472.

[50] Sherwood RI, Hashimoto T, O’Donnell CW, et al. Discovery of directional and nondirectional pioneer transcription factors by modeling DNase profile magnitude and shape. Nat Biotechnol. 2014;32:171–178.

[51] Nechaev S, Fargo DC, Dos Santos G, et al. Global analysis of short RNAs reveals widespread promoter-proximal stalling and arrest of Pol II in Drosophila. Science. 2010;327:335–338.

[52] Rennie S, Dalby M, Lloret-Llinares M, et al. Transcription start site analysis reveals widespread divergent transcription in D. melanogaster and core promoter-encoded enhancer activities. Nucleic Acids Res. 2018;46:5455–5469.

[53] Meers MP, Adelman K, Duronio RJ, et al. Transcription start site profiling uncovers divergent transcription and enhancer-associated RNAs in Drosophila melanogaster. BMC Genomics. 2018;19:157.

[54] Hetzel J, Duttke SH, Benner C, et al. Nascent RNA sequencing reveals distinct features in plant transcription. Proc Natl Acad Sci U S A. 2016;113:12316–12321.

[55] Zhu J, Liu M, Liu X, et al. RNA polymerase II activity revealed by GRO-seq and pNET-seq in Arabidopsis. Nat Plants. 2018;4:1112–1123.

[56] Feller A, Machemer K, Braun EL, et al. Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. Plant J. 2011;66:94–116.

[57] Davidson CJ, Guthrie EE, Lipsick JS. Duplication and maintenance of the Myb genes of vertebrate animals. Biol Open. 2013;2:101–110.

[58] Ambawat S, Sharma P, Yadav NR, et al. MYB transcription factor genes as regulators for plant responses: an overview. Physiol Mol Biol Plants. 2013;19:307–321.

[59] Kumamoto AA, Miller WG, Gunsalus RP. Escherichia coli tryptophan repressor binds multiple sites within the aroH and trp operators. Genes Dev. 1987;1:55–564.

[60] Bell SD, Cairns SS, Robson RL, et al. Transcriptional regulation of an archaeal operon in vivo and in vitro. Mol Cell. 1999;4:971–982.

[61] Brinkman AB, Dahlke I, Tuininga JE, et al. An Lrp-like transcriptional regulator from the archaeon Pyrococcus furiosus is negatively autoregulated. J Biol Chem. 2000;275:38160–38169.

[62] Vierke G, Engelmann A, Hebbeln C, et al. A novel archaeal transcriptional regulator of heat shock response. J Biol Chem. 2003;278:18–26.

[63] Getman DK, Mutero A, Inoue K, et al. Transcription factor repression and activation of the human acetylcholinesterase gene. J Biol Chem. 1995;270:23511–23519.

[64] Sakai DD, Helms S, Carlstedt-Duke J, et al. Hormone-mediated repression: a negative glucocorticoid response element from the bovine prolactin gene. Genes Dev. 1998;2:1144–1154.

[65] Meyer BJ, Kleid DG, Ptashne M. Lambda repressor turns off transcription of its own gene. Proc Natl Acad Sci U S A. 1975;72:4785–4789.

[66] Johnson A, Meyer BJ, Ptashne M. Mechanism of action of the cro protein of bacteriophage lambda. Proc Natl Acad Sci U S A. 1978;75:1783–1787.

[67] Myers RM, Rio DC, Robbins AK, et al. SV40 transcription is negatively autoregulated by the cooperative binding of T antigen to DNA. Cell. 1981;25:373–384.

[68] Kato H, Horikoshi M, Roeder RG. Repression of HIV-1 transcription by a cellular protein. Science. 1991;251:1476–1479.