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Bacterial Transcription Factors: Regulation by Pick 'n' Mix

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Highlights:

- Bacterial transcription factor activity is modulated by environmental signals
- Bacterial transcription factors bind at or near specific promoters and up- or down-regulate transcript initiation
- At many promoters, combinations of transcription factors work together to integrate different signals
- At many promoters, transcription factors work together with other DNA-binding proteins whose primary role is to sculpt the bacterial folded chromosome

Keywords: Bacterial Transcription, RNA Polymerase, Promoters, Transcription Activators, Transcription Repressors

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Abstract

Transcription in most bacteria is tightly regulated in order to facilitate bacterial adaptation to different environments, and transcription factors play a key role in this. Here we give a brief overview of the essential features of bacterial transcription factors and how they affect transcript initiation at target promoters. We focus on complex promoters that are regulated by combinations of activators and repressors, combinations of repressors only, or combinations of activators. At some promoters, transcript initiation is regulated by nucleoid-associated proteins, which often work together with transcription factors. We argue that the distinction between nucleoid-associated proteins and transcription factors is blurred and that they likely share common origins.

Introduction

Although a host of different protein factors work together with the bacterial DNA-dependent RNA polymerase (RNAP) to orchestrate the production of bacterial transcripts, here we restrict the discussion to proteins that interact at one or more specific promoters, either to repress or activate transcript initiation. The pioneering work of Francois Jacob and Jacques Monod set the scene with the identification of the *Escherichia coli* K-12 lactose operon repressor that controls lactose-induced activation of the operon, and the bacteriophage lambda cI repressor protein that maintains the quiescence of the integrated lambda prophage in lysogens [1]. These factors, together with the AraC and cyclic AMP receptor activator proteins, discovered soon after, became the cornerstone of developments to the operon hypothesis, in which specific environmental signals toggled the activity of specific promoters to enable adaptation [2]. Here we give a brief overview of our current understanding about bacterial transcription factor networks, focussing on complex regulatory regions containing promoters that are regulated by two or more different factors. The activity of most bacterial transcription factors is controlled by just one environmental signal. Hence, complex regulatory regions function as integrators for different environmental inputs [3, 4].

Basic functions of transcription factors

Transcription factor function depends on ability to recognise specific DNA sequences at target promoters and then either up- or down-regulate transcript initiation at that promoter [5]. For most factors, an independently-folding domain carries the DNA-binding motif, but this is rarely enough to confer sufficient specificity, and so most factors are functional as dimers (Fig. 1). An interesting exception is found with members of the AraC family, where a signature ~100 amino acid domain carries two independent helix-turn-helix motifs that, together, can create sufficient specificity [6]. For many transcription factors, a second independently folded domain is responsible for regulation. Interplay between the DNA-binding domain and the regulatory domain then modulates transcription factor activity, triggered by ligand binding, covalent modification or interaction with another protein [7] (Fig. 1). For transcription factors that lack a regulatory domain, their activity tends to be set by their availability for binding at targets that, often, is fixed by their level of expression.

Most bacterial transcription factors that function as repressors bind to DNA targets that overlap essential elements at their target promoters, thereby occluding access of RNAP (Fig. 2a) [8, 9]. In many cases, repression is enhanced by multiple binding of repressor molecules, which at some promoters, bind distally to each other and interact with each other via DNA loops (Fig. 2b). At other

promoters subject to repression, RNAP is able to engage but is blocked at the promoter by the repressor (Fig. 2c).

Most bacterial transcription factors that function as activators bind to DNA targets located just upstream of the essential elements at their target promoters [10]. Such factors often interact with RNAP and this results in its recruitment to the target promoter, thereby increasing transcript initiation (Fig. 2d) [11]. The activation surface on the factor, known as the Activating Region, is usually comprised of a small cluster of amino acid sidechains that make direct contact with a cognate surface somewhere on RNAP, usually on Domain 4 of the RNAP sigma subunit or the C-terminal domain of the RNAP alpha subunit. Other activators induce conformation changes in promoter DNA that result in adjustment in the spacing between different essential elements such that they can be served by RNAP (Fig. 2e) [12]. For the majority of activators that function at promoters served by RNAP carrying the 'housekeeping' sigma factor (or one related to the housekeeping sigma), transcript activation occurs without any major conformation change in the RNAP. However, for RNAP carrying a sigma factor related to Sigma-54, this is not the case, as major conformation changes are required for transcript initiation [13]. These conformation changes are driven by activator-RNAP interactions energised by ATP hydrolysis by a special class of activators known as Enhancer-binding Proteins (EBPs: see [14]).

Molecular analysis of the regulatory regions of many bacterial transcription units has shown that they are often not simple, with the involvement of many different transcription factors [15]. Since the activity of most bacterial transcription factors is regulated by just one signal, we can regard bacterial promoters as integration devices converting messages from the different factors into a single output. Hence, here, we consider three classes of promoters: those controlled by both an activator and a repressor, those controlled just by repressors, and those controlled by two activators.

Complex promoters: activators and repressors

The simplest activator-repressor scenario is when a promoter that is activated by one transcription factor is also repressed by another factor that binds independently. This is the situation at the *E. coli* K-12 lactose (*lac*) operon promoter whose activity is dependent on the cyclic AMP receptor protein (CRP), but is repressed by binding of the lactose operon repressor (LacI) (Fig. 3a). CRP binds to a DNA target centred between positions 61 and 62 upstream from the transcript start (denoted position -61.5) whilst LacI binds to a target centred 11 base pairs downstream from the transcript start (denoted position +11) [16, 17]. This arrangement, with an independently binding activator and repressor, can be found at many bacterial promoters and ensures the integration of two metabolic signals. Although CRP and the LacI repressor function independently at the *lac* promoter, there is a complication as LacI binding to secondary sites downstream at +412 and upstream at position -82 reinforce repression, possibly by forming a LacI tetramer-mediated loop between LacI dimers bound at different targets [18, 19]. The loop between the LacI operator sites at position -82 and +11 includes the DNA site for CRP, and LacI-CRP interactions are possible, though their significance is unclear. Clearer examples of functional Repressor-Activator direct interactions can be found at promoters that are repressed by CytR, a LacI family member regulated by the binding of Cytidine. Most of these promoters are dependent on activation by CRP, and repressive binding of CytR requires two prebound CRP molecules (Fig. 3b) [20]. An interesting variation is seen at promoters involving transcription factors that can function both as activators and repressors. Usually the first

bound molecule, which occupies the DNA site with the highest affinity, acts as an activator, whilst subsequent factor binding causes repression (Fig. 3c) [21].

Complex promoters: two repressors

The simplest scenario for a promoter involving two different repressors is that the two repressors bind independently. This arrangement is found with some promoters that are repressed by LexA or related repressors, which regulate bacterial responses to DNA damage (the SOS response) and other extreme stresses that indirectly damage DNA [22, 23]. In combination with LexA, the second repressor enables the host bacterium to delay the expression of certain lethal gene products, thereby uncoupling their expression from the temporal induction of DNA repair genes that are controlled only by LexA [24, 25]. Thus the promoter of the *E. coli* gene encoding the pore-forming bacteriocin, colicin K, is repressed by LexA and also by IscR (which is induced by oxidative stress), and, hence colicin K expression is double-locked, requiring two signals for full induction, but neither transcription factor is sufficient for full repression (Fig. 4a) [26]. Alternatively, the binding of a repressor can be enhanced by interaction with a co-repressor. For example, genes involved in the replication and regulation of the temperate *Bacillus thuringiensis* GIL01 'phage are repressed by a complex of LexA and 'phage-encoded gp7 protein, and are induced by a single signal (Fig. 4b) [27]. Hence, at the GIL01 lysogenic P1 promoter, Gp7 directly interacts with LexA and increases repressor affinity for a non-canonical target site, thereby delaying the induction of the phage promoter compared to other LexA-repressed host promoters [27]. Another situation is seen with some of the genes encoding nuclease colicins whose promoters are repressed by a nucleoprotein complex involving LexA and AsnC (which is induced by asparagine depletion) (Fig. 4c) [28].

At many promoters, nucleoid-associated proteins (NAPs) are directly involved in repression of transcript initiation, either with or without a 'conventional' transcription factor [29]. Recall that most NAPs are small DNA binding proteins present in all bacteria and that their primary role is in sculpturing the folded chromosome [30]. They are often present in large quantities and bind DNA promiscuously and this mostly results in transcription repression. For example, H-NS forms arrays along DNA segments that result in loci that are distant on the genetic map being brought together and, often, silenced. However some abundant NAPs (e.g. Fis, the factor for inversion stimulation, and IHF, Integration host factor) do exhibit preferential binding at certain sites. Hence the *E. coli dps* promoter is regulated by Fis and H-NS, with Fis jamming RNAP at the promoter whilst H-NS blocks its access (Fig. 4d) [31].

Complex promoters: two activators

The simplest scenario is the 'either-or' scenario where either one or another activator can activate a target promoter, with either factor being deployed according to the growth environment [32, 33]. However, at most bacterial promoters regulated by two activators, activity is co-dependent on both activators and more complex mechanisms operate. The first of these to be described involved a promoter that could be fully activated by just one activator but this activator bound to the promoter at a location where it was unable to activate [34]. Subsequent binding of the second activator repositioned the first, principal activator from a location where it was unable to activate to a location where it could activate (Fig. 5a). A variation of this is found at promoters where DNA bending is required to bring an upstream-bound activator into contact with its target RNAP [35]. This arrangement is found at many promoters served by RNAP containing sigma-54, where IHF-induced

bending is essential to facilitate direct interaction between an upstream-bound EBP and the RNAP (Fig. 5a) [14].

For a relatively small number of promoters, co-dependence on two activators is due to a requirement for cooperative interactions between the activators for target binding (Fig. 5b). Hence, at the *E. coli* K-12 *melAB* promoter, cooperative binding of CRP and MelR is essential for MelR to be able to recruit RNAP to the promoter [36]. Similarly at CRP-dependent promoters carrying a certain variation of the consensus DNA target sequence for CRP, a co-activator, Sxy, is required for CRP binding and promoter activation [37]. A more commonly found arrangement is for each of the two activators to bind independently at the target promoter and then make independent contacts with the RNAP, with both contacts being essential for RNAP recruitment (Fig. 5c) [38-40]. At such promoters, one of the activators binds at a location that is suboptimal for activation, and it is this that confers the co-dependence on both activators [41, 42].

Finally, many co-dependent promoters are, in fact, dependent just on one activator, the principal activator, but the action of this activator is suppressed, often by the action of NAPs. In these cases, the role of the second activator is to relieve this suppression (Fig. 5d) [29, 43]. We have previously argued that activation by reversing suppression by NAPs is an ancient activation mechanism [44], and, to illustrate this, below we present some case studies involving NarL, a nitrate/nitrite-triggered response-regulator

Lessons from *E. coli* promoters dependent on NarL

E. coli NarL is a typical bacterial response-regulator, activated by sensor kinases that are activated by external nitrate or nitrite ions [45]. The Regulon DB database [46] lists 26 target promoters directly regulated by NarL, with 15 that are repressed and 11 that are activated. At the repressed promoters, NarL appears to act by blocking RNAP access to essential promoter elements. However, at the activated promoters, two distinct mechanisms operate, and we contrast these here by describing the regulatory regions of the *nir* operon, which encodes an NADH-dependent nitrite reductase, and the *yeaR-yoaG* operon, whose function, as yet, is unsure (Fig. 6).

Expression of the *nir* operon is due to activation of a single promoter in response to two environmental signals, the absence of oxygen, mediated by FNR protein, and the presence of nitrate or nitrite ions, mediated by NarL. FNR binds to a single target at position -41.5 and can fully activate the promoter by recruitment of RNAP (Fig. 6a) [43]. However, the binding of several upstream NAPs, Fis, IHF, and H-NS, suppresses this activation, but nitrate/nitrite-induced binding of NarL to a single target site located at position -69.5 reverses this suppression (Fig. 6a) [47-49]. Effectively, the primary role of NarL is to disrupt the local NAP organisation to permit FNR function. The situation is somewhat different at the *yeaR-yoaG* operon promoter where NarL binds to a target located at position -43.5 and activates transcription via an activating region that makes a direct interaction that recruits RNAP to the promoter (Fig. 6b) [50, 51]. We have dubbed these distinct mechanisms as ‘ancient’ and ‘modern’ because, in bacteria, NAPs appear to be more ancient than transcription factors because DNA compaction is essential whilst transcription regulation is not [44]. Thus, we imagine that the first transcription activators achieved their purpose simply by pushing NAPs out of the way, as at the *E. coli nir* promoter, whilst activating regions evolved later as precision contacts that recruited and positioned RNAP to enable transcript initiation at targeted promoters.

Further studies of both the *nir* and *yeaR-yoaG* regulatory regions revealed other interesting features that echo the points outlined above. For example, even at the *yeaR* promoter, NarL has to overcome

some NAP-mediated repression. This is because Fis binds to a target site that overlaps the DNA site for NarL and this sets a threshold level for active NarL needed to displace Fis and activate the promoter [52]. Similarly, at the *nir* promoter, NarL must directly displace a NAP, which is IHF bound at position -88 [48], but a second IHF, bound just upstream, at position -115, is unaffected by NarL, and, surprisingly, this bound IHF activates rather than represses FNR-triggered *nir* operon transcription (Fig. 6a) [49]. Comparison of *nir* operon regulatory region sequences from different *E. coli* strains and related bacteria suggests a biological role for this. In anaerobic conditions, in the absence of nitrate or nitrite ions, the balance between activation by IHF bound at position -115 and IHF, Fis and H-NS bound at the other sites, sets the basal level of NarL-independent *nir* operon expression.

Another feature of both the *nir* and *yeaR* promoter regions is that they can be shut down by a repressor that can 'overrule' all the activatory input from NarL (and FNR). Hence, the *yeaR* promoter is shut down by the NsrR repressor that binds to a target at position -32 (Fig. 6b) [50, 53]. Since NsrR is induced by reactive nitrogen species (RNS), this argues that the function of the YeaR and YoaG gene products may be in protection from, or recovery from, RNS exposure. Similarly, the *nir* operon promoter is inhibited by the binding of Cra repressor (also known as FruR) to a target at position -16.5 (Fig. 6a) [54]. Since Cra (FruR) is induced by high levels of glycolytic intermediates, *nir* expression is restricted to cells growing in relatively rich media.

Perspectives

It has previously been suggested that the different mechanisms for the regulation of bacterial transcript initiation fall into two classes, those that are focussed on the RNA polymerase and those focused on target promoters, reflecting two distinct regulatory strategies [55]. RNAP-focussed strategies include alternative sigma factors, small ligands such as ppGpp, and RNAP helpers, blockers and appropriators. Top of the list of promoter-focussed mechanisms are the transcription factors discussed here and nucleoid-associated proteins. As illustrated above, some bacterial promoters have evolved to exploit combinations of transcription factors, and, at many of these, NAPs are also involved. It has been argued that NAPs must be more ancient than transcription factors, since chromosome folding and compaction is essential whilst regulation is not [56]. The observation that some NAPs can act as transcription factors, stimulating or repressing transcript initiation at specific promoters, has prompted the suggestion that bacterial transcription factors may have evolved from NAPs, and this may explain why some gene regulatory proteins classed as transcription factors display many properties associated with NAPs. Thus, though our knowledge is still quite limited since the properties of the vast majority of bacterial gene regulatory proteins have yet to be investigated, we propose that there exists a continuum between bacterial NAPs and transcription factors and the existing division between them is inappropriate. In any case, the stunning diversity of observed organisations at bacterial promoters, discovered during the six decades that have elapsed since the Jacob-Monod operon model, suggests that evolution will eventually find a way to circumvent any classification system that we invent!

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References

[1] F. Jacob, La statue intérieure. Éditions Odile Jacob, Paris 1987.
[2] B. Müller-Hill, The *lac* Operon. A Short History of a Genetic Paradigm. Walter de Gruyter, Berlin New York 1996.
[3] S. Baumberg, Prokaryotic Gene Expression. Oxford University Press, Oxford 1999.
[4] A.S.N. Seshasayee, K. Sivaraman, N.M. Luscombe, An Overview of Prokaryotic Transcription Factors. in: T.R. Hughes (Ed.). A Handbook of Transcription Factors. Springer, Dordrecht 2011. pp. 7-23.
[5] E. Perez-Rueda, R. Hernandez-Guerrero, M.A. Martinez-Nunez, D. Armenta-Medina, I. Sanchez, J.A. Ibarra, Abundance, diversity and domain architecture variability in prokaryotic DNA-binding transcription factors, PLoS One. 13 (2018) e0195332.
[6] M.T. Gallegos, R. Schleif, A. Bairoch, K. Hofmann, J.L. Ramos, Arac/XylS family of transcriptional regulators, Microbiol Mol Biol Rev. 61 (1997) 393-410.
[7] D.F. Browning, S.J. Busby, The regulation of bacterial transcription initiation, Nat Rev Microbiol. 2 (2004) 57-65.
[8] L. Bintu, N.E. Buchler, H.G. Garcia, U. Gerland, T. Hwa, J. Kondev, et al., Transcriptional regulation by the numbers: models, Curr Opin Genet Dev. 15 (2005) 116-24.
[9] L. Bintu, N.E. Buchler, H.G. Garcia, U. Gerland, T. Hwa, J. Kondev, et al., Transcriptional regulation by the numbers: applications, Curr Opin Genet Dev. 15 (2005) 125-35.
[10] D.J. Lee, S.D. Minchin, S.J. Busby, Activating transcription in bacteria, Annu Rev Microbiol. 66 (2012) 125-52.
[11] S. Busby, R.H. Ebright, Promoter structure, promoter recognition, and transcription activation in prokaryotes, Cell. 79 (1994) 743-6.
[12] N.L. Brown, J.V. Stoyanov, S.P. Kidd, J.L. Hobman, The MerR family of transcriptional regulators, FEMS Microbiol Rev. 27 (2003) 145-63.
[13] Y. Yang, V.C. Darbari, N. Zhang, D. Lu, R. Glyde, Y.P. Wang, et al., Structures of the RNA polymerase-sigma54 reveal new and conserved regulatory strategies, Science. 349 (2015) 882-5.
[14] M. Buck, M.T. Gallegos, D.J. Studholme, Y. Guo, J.D. Gralla, The bacterial enhancer-dependent sigma(54) (sigma(N)) transcription factor, J Bacteriol. 182 (2000) 4129-36.
[15] A. Barnard, A. Wolfe, S. Busby, Regulation at complex bacterial promoters: how bacteria use different promoter organizations to produce different regulatory outcomes, Curr Opin Microbiol. 7 (2004) 102-8.
[16] C.J. Wilson, H. Zhan, L. Swint-Kruse, K.S. Matthews, The lactose repressor system: paradigms for regulation, allosteric behavior and protein folding, Cell Mol Life Sci. 64 (2007) 3-16.
[17] B. Liu, C. Hong, R.K. Huang, Z. Yu, T.A. Steitz, Structural basis of bacterial transcription activation, Science. 358 (2017) 947-51.
[18] S. Oehler, E.R. Eismann, H. Kramer, B. Muller-Hill, The three operators of the *lac* operon cooperate in repression, Embo J. 9 (1990) 973-9.
[19] D.F. Browning, K.L. Richards, R.E. Godfrey, C. Robinson, S.J.W. Busby, Exploitation of the *Escherichia coli lac*-operon promoter for controlled recombinant protein production, Biochemical Society Transactions. (2019) In press.
[20] P. Valentin-Hansen, L. Sogaard-Andersen, H. Pedersen, A flexible partnership: the CytR anti-activator and the cAMP-CRP activator protein, comrades in transcription control, Mol Microbiol. 20 (1996) 461-6.
[21] K.B. Decker, T.D. James, S. Stibitz, D.M. Hinton, The *Bordetella pertussis* model of exquisite gene control by the global transcription factor BvgA, Microbiology. 158 (2012) 1665-76.
[22] M. Butala, D. Zgur-Bertok, S.J. Busby, The bacterial LexA transcriptional repressor, Cell Mol Life Sci. 66 (2009) 82-93.
[23] A. Aertsen, C.W. Michiels, Upstream of the SOS response: figure out the trigger, Trends Microbiol. 14 (2006) 421-3.

[24] J. Courcelle, A. Khodursky, B. Peter, P.O. Brown, P.C. Hanawalt, Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient *Escherichia coli*, Genetics. 158 (2001) 41-64.
[25] M.J. Culyba, J.M. Kubiak, C.Y. Mo, M. Goulian, R.M. Kohli, Non-equilibrium repressor binding kinetics link DNA damage dose to transcriptional timing within the SOS gene network, PLoS Genet. 14 (2018) e1007405.
[26] M. Butala, S. Sonjak, S. Kamensek, M. Hodoscek, D.F. Browning, D. Zgur-Bertok, et al., Double locking of an *Escherichia coli* promoter by two repressors prevents premature colicin expression and cell lysis, Mol Microbiol. 86 (2012) 129-39.
[27] N. Fornelos, M. Butala, V. Hodnik, G. Anderluh, J.K. Bamford, M. Salas, Bacteriophage GIL01 gp7 interacts with host LexA repressor to enhance DNA binding and inhibit RecA-mediated auto-cleavage, Nucleic Acids Res. 43 (2015) 7315-29.
[28] S. Kamensek, D.F. Browning, Z. Podlesek, S.J. Busby, D. Zgur-Bertok, M. Butala, Silencing of DNase colicin E8 gene expression by a complex nucleoprotein assembly ensures timely colicin induction, PLoS Genet. 11 (2015) e1005354.
[29] D.F. Browning, D.C. Grainger, S.J. Busby, Effects of nucleoid-associated proteins on bacterial chromosome structure and gene expression, Curr Opin Microbiol. 13 (2010) 773-80.
[30] S.C. Dillon, C.J. Dorman, Bacterial nucleoid-associated proteins, nucleoid structure and gene expression, Nat Rev Microbiol. 8 (2010) 185-95.
[31] D.C. Grainger, M.D. Goldberg, D.J. Lee, S.J. Busby, Selective repression by Fis and H-NS at the *Escherichia coli dps* promoter, Mol Microbiol. 68 (2008) 1366-77.
[32] K. Eiglmeier, N. Honore, S. Iuchi, E.C. Lin, S.T. Cole, Molecular genetic analysis of FNR-dependent promoters, Mol Microbiol. 3 (1989) 869-78.
[33] A.I. Bell, K.L. Gaston, J.A. Cole, S.J. Busby, Cloning of binding sequences for the *Escherichia coli* transcription activators, FNR and CRP: location of bases involved in discrimination between FNR and CRP, Nucleic Acids Res. 17 (1989) 3865-74.
[34] E. Richet, D. Vidal-Ingigliardi, O. Raibaud, A new mechanism for coactivation of transcription initiation: repositioning of an activator triggered by the binding of a second activator, Cell. 66 (1991) 1185-95.
[35] I. Schroder, S. Darie, R.P. Gunsalus, Activation of the *Escherichia coli* nitrate reductase (*narGHJI*) operon by NarL and Fnr requires integration host factor, J Biol Chem. 268 (1993) 771-4.
[36] J.T. Wade, T.A. Belyaeva, E.I. Hyde, S.J. Busby, A simple mechanism for co-dependence on two activators at an *Escherichia coli* promoter, Embo J. 20 (2001) 7160-7.
[37] M. Jaskolska, K. Gerdes, CRP-dependent positive autoregulation and proteolytic degradation regulate competence activator Sxy of *Escherichia coli*, Mol Microbiol. 95 (2015) 833-45.
[38] J.K. Joung, D.M. Koepp, A. Hochschild, Synergistic activation of transcription by bacteriophage lambda cI protein and *E. coli* cAMP receptor protein, Science. 265 (1994) 1863-6.
[39] S. Scott, S. Busby, I. Beacham, Transcriptional co-activation at the *ansB* promoters: involvement of the activating regions of CRP and FNR when bound in tandem, Mol Microbiol. 18 (1995) 521-31.
[40] C.M. Beatty, D.F. Browning, S.J. Busby, A.J. Wolfe, Cyclic AMP receptor protein-dependent activation of the *Escherichia coli acsP2* promoter by a synergistic class III mechanism, J Bacteriol. 185 (2003) 5148-57.
[41] A.E. Rossiter, D.F. Browning, D.L. Leyton, M.D. Johnson, R.E. Godfrey, C.A. Wardius, et al., Transcription of the plasmid-encoded toxin gene from enteroaggregative *Escherichia coli* is regulated by a novel co-activation mechanism involving CRP and Fis, Mol Microbiol. 81 (2011) 179-91.
[42] A.E. Rossiter, R.E. Godfrey, J.A. Connolly, S.J. Busby, I.R. Henderson, D.F. Browning, Expression of different bacterial cytotoxins is controlled by two global transcription factors, CRP and Fis, that co-operate in a shared-recruitment mechanism, Biochem J. 466 (2015) 323-35.
[43] H. Wu, K.L. Tyson, J.A. Cole, S.J. Busby, Regulation of transcription initiation at the *Escherichia coli nir* operon promoter: a new mechanism to account for co-dependence on two transcription factors, Mol Microbiol. 27 (1998) 493-505.

[44] S.J.W. Busby, Transcription activation in bacteria: ancient and modern, Microbiology. (2019) doi: 10.1099/mic.0.000783. [Epub ahead of print].
[45] A.J. Darwin, V. Stewart, The NAR Modulon Systems: Nitrate and Nitrite Regulation of Anaerobic Gene Expression. in: E.C.C. Lin, A.S. Lynch (Eds.), Regulation of Gene Expression in *Escherichia coli*. Springer, Boston, MA 1996. pp. 343-59
[46] A. Santos-Zavaleta, M. Sanchez-Perez, H. Salgado, D.A. Velazquez-Ramirez, S. Gama-Castro, V.H. Tierrafria, et al., A unified resource for transcriptional regulation in *Escherichia coli* K-12 incorporating high-throughput-generated binding data into RegulonDB version 10.0, BMC Biol. 16 (2018) 91.
[47] D.F. Browning, J.A. Cole, S.J. Busby, Suppression of FNR-dependent transcription activation at the *Escherichia coli nir* promoter by Fis, IHF and H-NS: modulation of transcription initiation by a complex nucleo-protein assembly, Mol Microbiol. 37 (2000) 1258-69.
[48] D.F. Browning, J.A. Cole, S.J. Busby, Transcription activation by remodelling of a nucleoprotein assembly: the role of NarL at the FNR-dependent *Escherichia coli nir* promoter, Mol Microbiol. 53 (2004) 203-15.
[49] D.F. Browning, J.A. Cole, S.J. Busby, Regulation by nucleoid-associated proteins at the *Escherichia coli nir* operon promoter, J Bacteriol. 190 (2008) 7258-67.
[50] H.Y. Lin, P.J. Bledsoe, V. Stewart, Activation of *yeaR-yoaG* operon transcription by the nitrate-responsive regulator NarL is independent of oxygen- responsive regulator Fnr in *Escherichia coli* K-12, J Bacteriol. 189 (2007) 7539-48.
[51] A.V. Lin, V. Stewart, Functional roles for the GerE-family carboxyl-terminal domains of nitrate response regulators NarL and NarP of *Escherichia coli* K-12, Microbiology. 156 (2010) 2933-43.
[52] D.J. Squire, M. Xu, J.A. Cole, S.J. Busby, D.F. Browning, Competition between NarL-dependent activation and Fis-dependent repression controls expression from the *Escherichia coli yeaR* and *ogt* promoters, Biochem J. 420 (2009) 249-57.
[53] N. Filenko, S. Spiro, D.F. Browning, D. Squire, T.W. Overton, J. Cole, et al., The NsrR regulon of *Escherichia coli* K-12 includes genes encoding the hybrid cluster protein and the periplasmic, respiratory nitrite reductase, J Bacteriol. 189 (2007) 4410-7.
[54] K. Tyson, S. Busby, J. Cole, Catabolite regulation of two *Escherichia coli* operons encoding nitrite reductases: role of the Cra protein, Arch Microbiol. 168 (1997) 240-4.
[55] D.F. Browning, S.J. Busby, Local and global regulation of transcription initiation in bacteria, Nat Rev Microbiol. 14 (2016) 638-50.
[56] S.S. Visweswariah, S.J. Busby, Evolution of bacterial transcription factors: how proteins take on new tasks, but do not always stop doing the old ones, Trends Microbiol. 23 (2015) 463-7.

Figure Legends

Figure 1: Key features of bacterial transcription factors

A transcription factor subunit is shown as a dumbbell shape with the two lobes denoting the Regulatory Domain (RD) and DNA-binding Domain (DBD). Two subunits dimerise and then interact with a bacterial promoter region (denoted as a horizontal line) to either Repress or Activate transcript initiation. RNAP is denoted as a cartoon multisubunit assembly (explained in Fig. 2 legend) that will initiate transcription at the point denoted by the start of a bent arrow, with a cross denoting blockage of initiation in the Repression case. Dotted arrows converging on the transcription factor RD indicate the different ways that transcription factor activity can be modulated.

Figure 2: Mechanisms of repression and activation by transcription factors at bacterial promoters

In each panel, the target promoter region is shown as a line with different promoter elements shown by rectangles, and the transcript start, marked +1, indicated with a bent arrow that shows the direction of transcription. Transcription factors are shown as circular or oval dimers. The multisubunit RNAP is sketched, as in Fig. 1, with the two catalytic subunits, β and β', drawn as a larger oval, the sigma subunit drawn as a smaller darker-shaded oval, and each of the two α subunits drawn as a dumbbell with a curved line to illustrate the flexible linker between the N- and C-terminal domains (illustrated by the two lobes of each dumbbell).

a) A repressor binds adjacent to key promoter elements and prevents RNAP engagement.
b) Repressor dimers bind at some distance from promoter elements but interact, thereby preventing RNAP access to the promoter.
c) RNAP binds to the promoter but is jammed by repressor binding.
d) Activator provides direct contact (small circle) with RNAP thereby recruiting RNAP to the promoter and facilitating transcript initiation.
e) Activator alters the juxtaposition of essential promoter elements so as to enable RNAP binding and subsequent transcript initiation.

Figure 3: Regulation at complex promoters: how repressors and activators interact

Promoters, transcription factors and RNAP are drawn using the same conventions as in Fig. 2.

a) The *E. coli lac* operon promoter is regulated by the repressor, LacI, and the activator, CRP, that bind independently.
b) Some CRP-activated promoters are repressed by CytR that makes a direct contact with CRP. Binding of CytR, hence repression by CytR, requires tandem-bound CRP dimers.
c) Binding of a transcription factor to its primary high affinity site activates transcript initiation at target promoter by a recruitment mechanism, but factor binding to a second lower affinity site causes repression.

Figure 4: Regulation at complex promoters: how repressors interact

Promoters, transcription factors and RNAP are drawn using the same conventions as in Fig. 2 but LexA is drawn as a dumbbell shape to depict its domain structure.

a) Repressors IscR and LexA bind to the promoter that controls expression of colicin K in *E. coli* and double lock the promoter.
b) The *Bacillus thuringiensis* GIL01 'phage P1 promoter is repressed by host LexA protein together with 'phage-encoded gp7 protein, which acts as a co-repressor.
c) The promoter that controls expression of DNase colicin E8 in *E. coli* is repressed by a multi-protein complex, which comprises of LexA and AsnC.
d) Nucleoid associated proteins repress the *E. coli dps* promoter: Fis jams RNAP at the promoter whilst H-NS occludes RNAP binding.

Figure 5: Regulation at complex promoters: how activators interact

Promoters, transcription factors and RNAP are drawn using the same conventions as in Fig. 2.

a) Activation by repositioning: (i) Activator 2 triggers the movement of Activator 1 from a site where it is unable to activate transcript initiation to one where it is able, (ii) Activator 2 induces DNA bending so that Activator 1 is able to make an interaction with promoter-bound RNAP that activates transcript initiation.
b) Activator binding to the target promoter requires co-operative interaction between Activator 1 and Activator 2. Binding results in activation of transcript initiation by RNAP recruitment.
c) Activator 1 and Activator 2 bind independently to the target promoter and each makes an independent contacts with RNAP that results in RNAP recruitment and activation of transcript initiation.
d) Activator 1 has the potential to activate transcription fully by an RNAP recruitment mechanism but its activity is suppressed by an upstream-bound repressor. Activator 2 blocks this suppression thereby activating the promoter.

Figure 6: Transcription activation by NarL by two contrasting mechanisms

Promoters and transcription factors are drawn using the same conventions as in Fig. 2 but RNAP is omitted for clarity.

a) The *E. coli nir* promoter: NarL counteracts the suppression of FNR-dependent activation by stopping the action of upstream-bound NAPs, Fis and IHF. To do this, it displaces IHF from the IHF I site. Note that the Cra repressor can override activation and shut down the promoter.
b) The *E. coli yeaR* promoter: NarL can activate transcript initiation by recruiting RNAP to the promoter. To do this, NarL must compete with upstream-bound Fis, but activation can be suppressed by NsrR.

Figure 1

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Figure 1

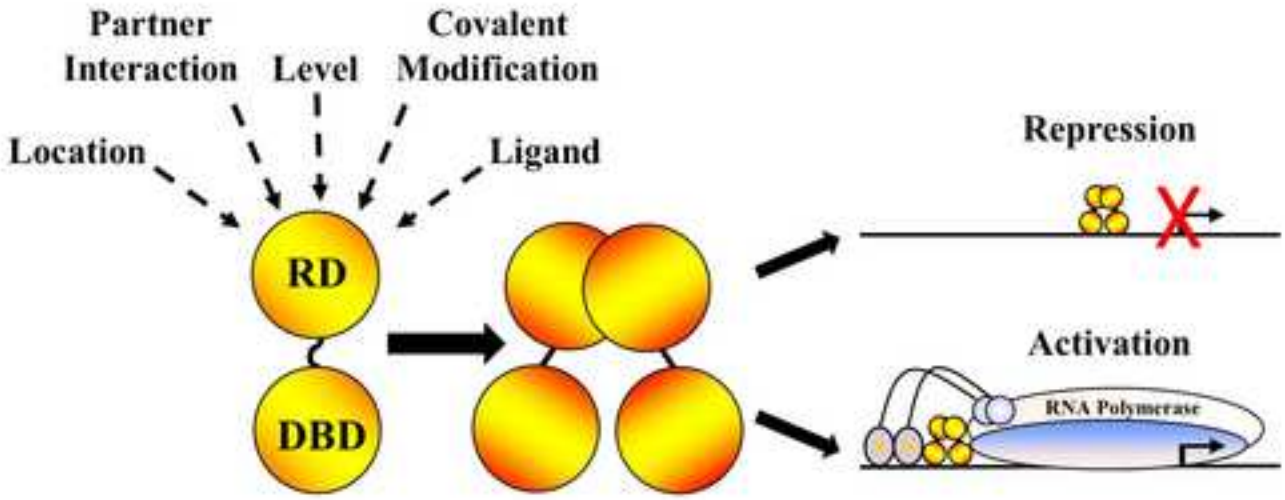

Figure 2
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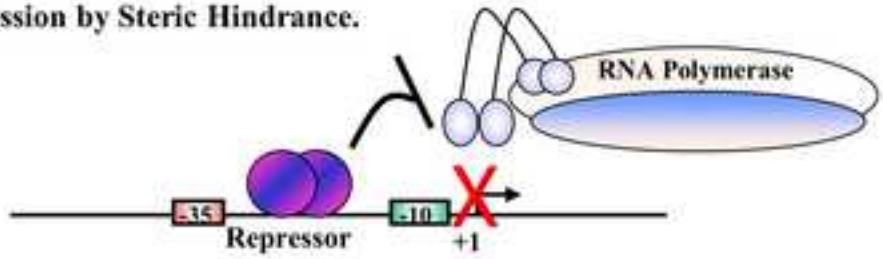
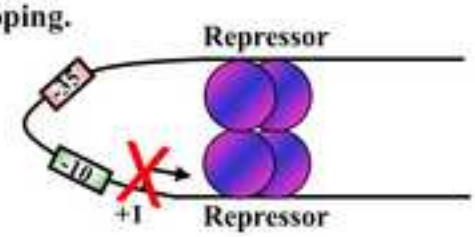
Figure 2.

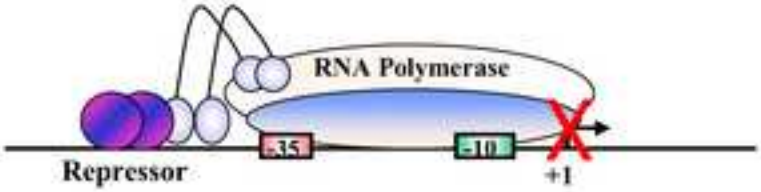
(a) Repression by Steric Hindrance.



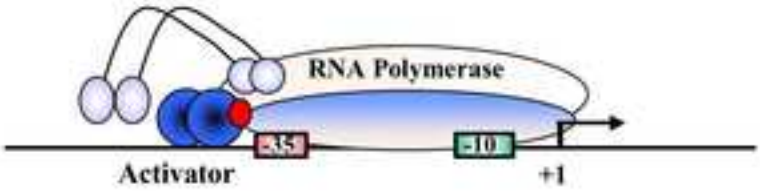
(b) Repression by Looping.



(c) Repression by Locking RNA Polymerase at a Promoter.



(d) Simple Activation.



(e) Activation by Conformation Change.

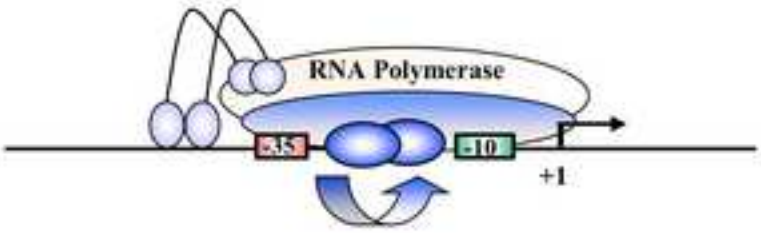


Figure 3
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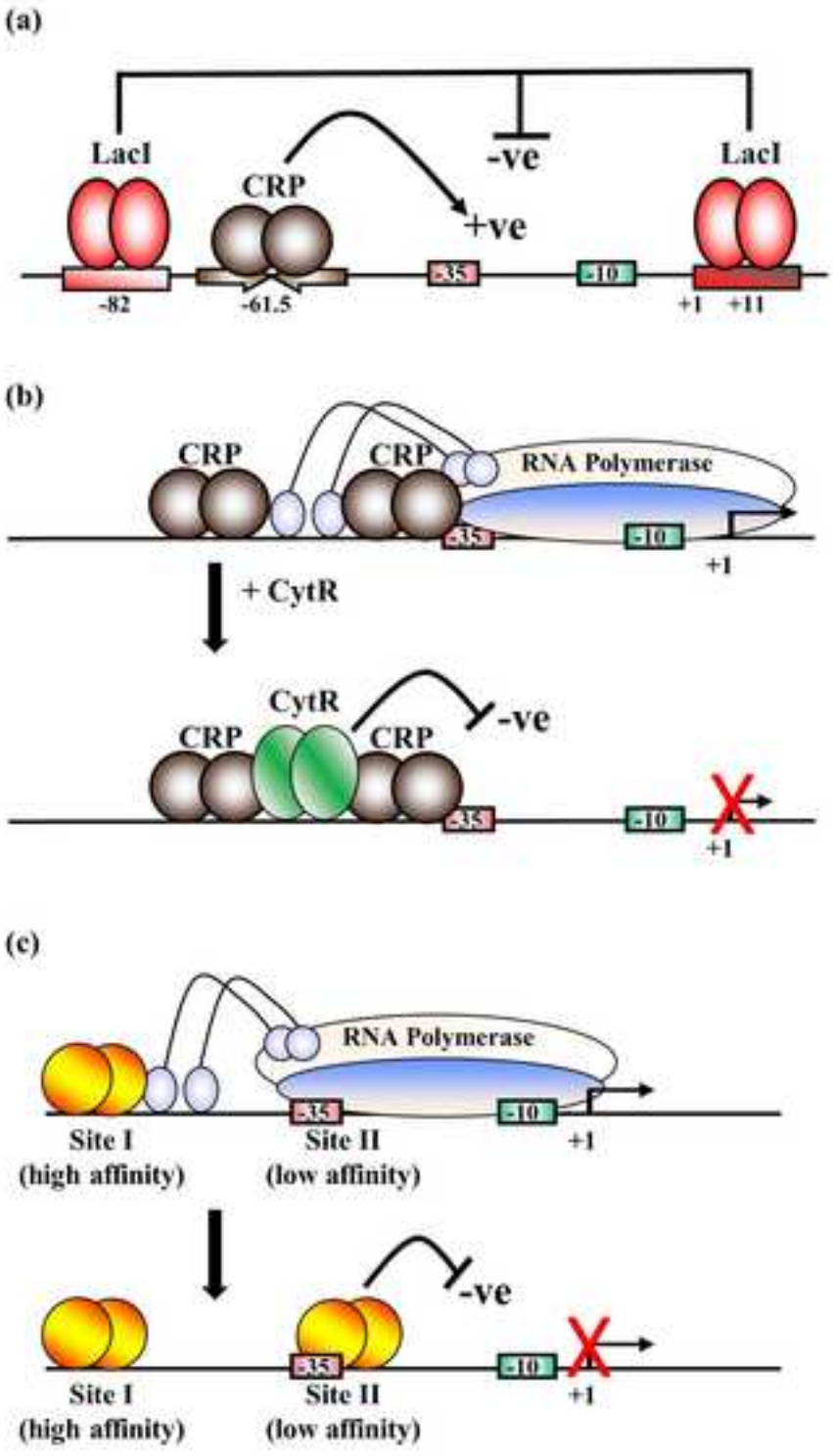

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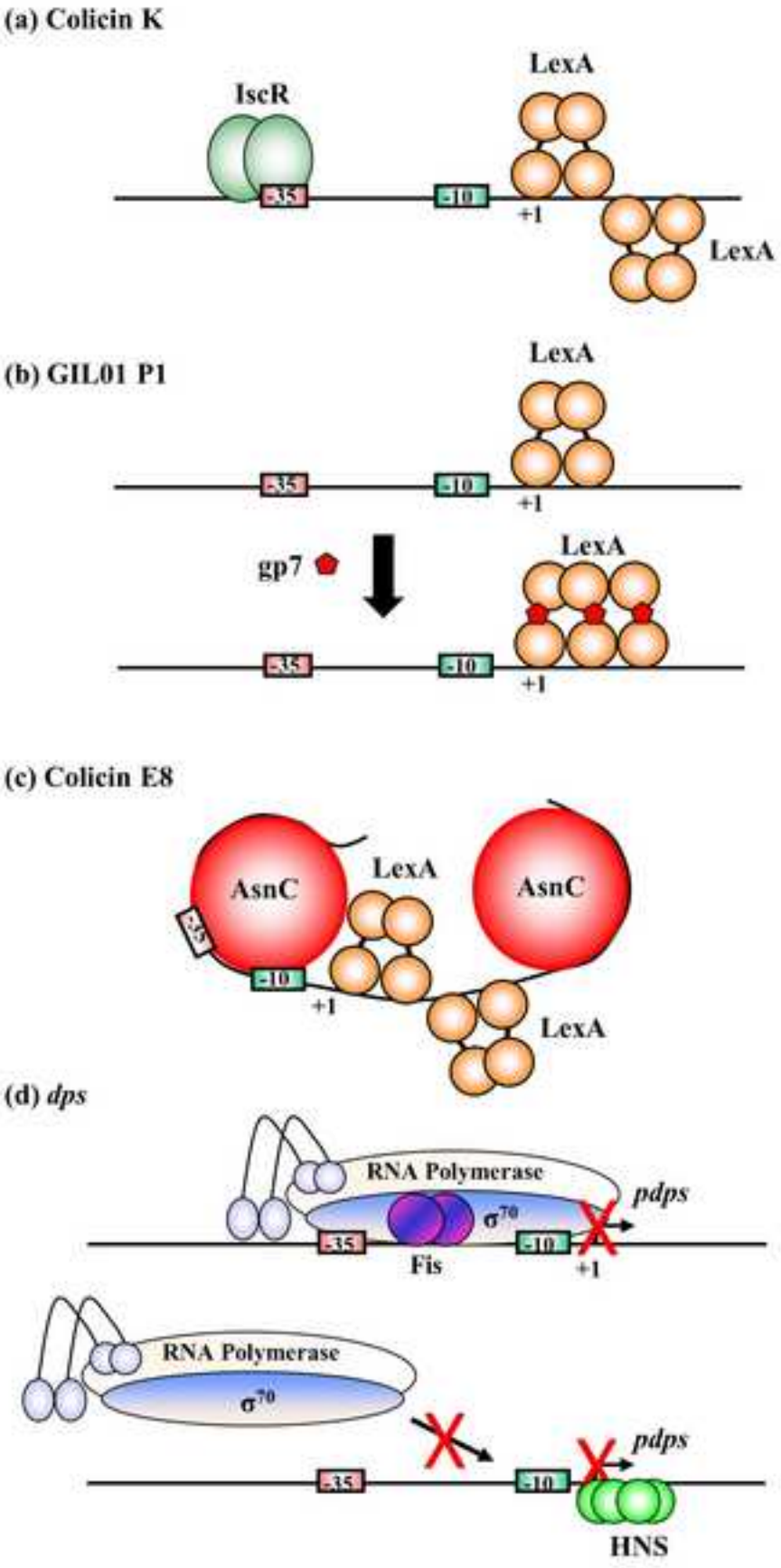

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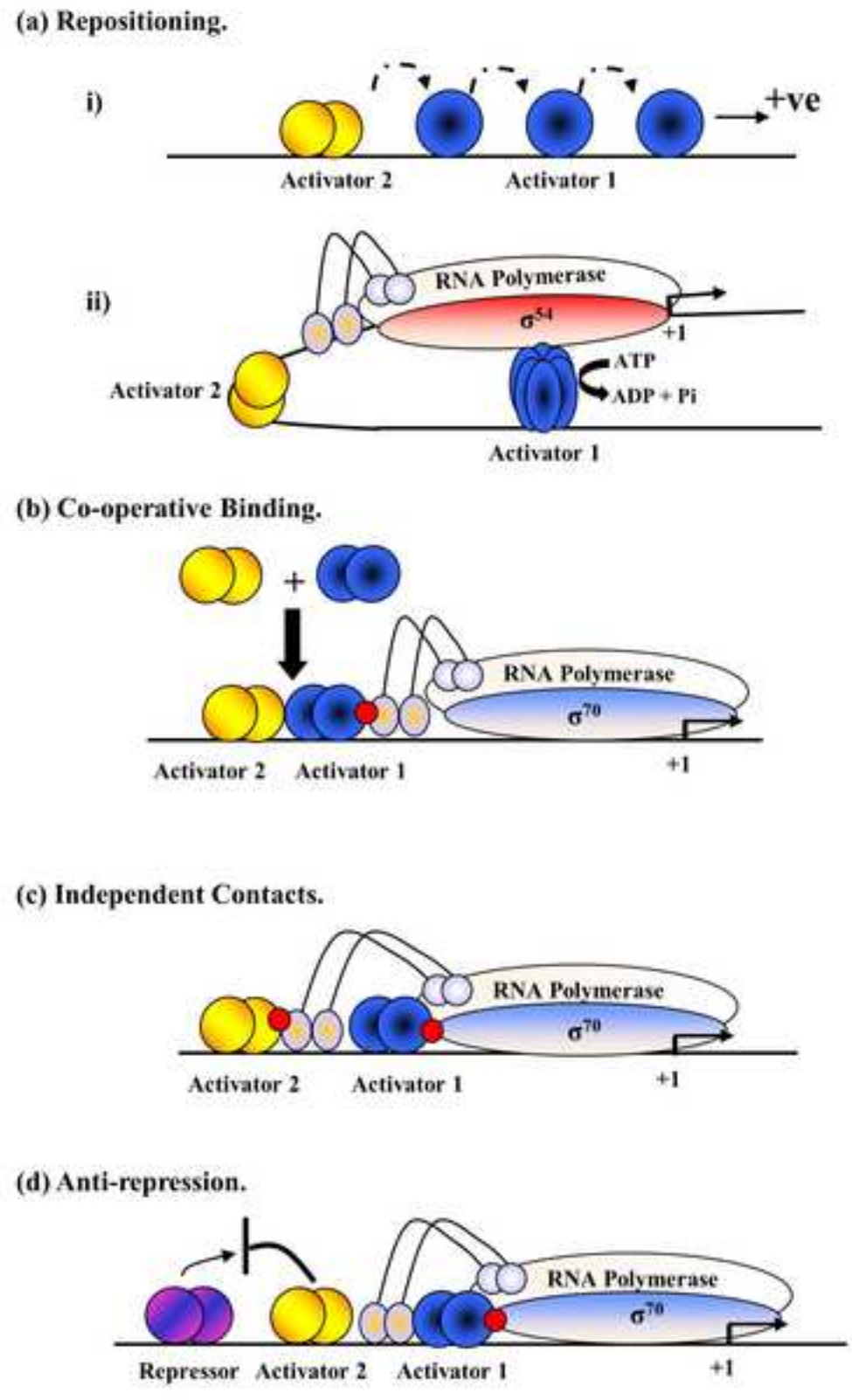

Figure 6
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