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Redefining fundamental concepts of transcription initiation in bacteria

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Abstract

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Despite enormous progress in understanding the fundamentals of bacterial gene regulation, our knowledge remains limited when compared with the number of bacterial genomes and regulatory systems to be discovered. Derived from a small number of initial studies, classic definitions for concepts of gene regulation have evolved as the number of characterized promoters has increased. Together with discoveries made by new technologies, this knowledge has led to revised generalizations and principles. In this Expert Recommendation, we suggest precise, updated definitions that support a logical, consistent, conceptual framework of bacterial gene regulation, focusing on transcription initiation. The resulting concepts can be formalized by ontologies for computational modelling, laying the foundation for improved bioinformatics tools, knowledge-based resources and scientific communication. Thus, this work will help to construct better predictive models, with different formalisms, that will be useful in engineering, synthetic biology, microbiology and genetics.

Introduction

Gene expression, the transcription of DNA into RNA and the translation of RNA into a polypeptide chain, and its regulation encompass a collection of genetic and molecular programmes that underlie the major biological capabilities of eukaryotic and prokaryotic cellular differentiation and development. Gene expression is regulated in response to environmental conditions, which is critical for bacterial fitness and survival. Any step in the gene expression pathway can be regulated, from transcription initiation to mRNA stability and translation. The foundations of our understanding of gene expression regulation rely on terminology and models derived from research in *Escherichia coli* and bacteriophage λ ^{1,2}. These fundamental studies have been followed by decades of research in a number of regulated bacterial systems and more recently by studies using high-throughput genomic methodologies and advanced biophysical single-molecule approaches^{3–5}, which have led to discoveries that could not have been imagined when the original concepts were proposed. Thus, some terms have acquired meanings that differ from their original ones. As experimental biology becomes a data science, mainly due to the advent of genomics, computational models that rigorously organize our knowledge become essential. Databases and ontologies are the two major tools that underpin the computational representation of knowledge. Because these tools are specified in formal language, they require definitions at a level of detail that is beyond what is common in communications among experts.

Here, we focus on transcription initiation, the most studied step in the regulation of gene expression. To discuss the limitations of existing definitions that constitute our core understanding of the regulation of transcription initiation in bacteria, the literature was searched for original and more recent definitions (Supplementary tables 1–5), as well as for examples of regulatory systems that do not conform to these definitions. A group of experts on the regulation of transcription initiation in bacteria organized a collective process of evaluating the necessity and sufficiency of the different features used to characterize the elements involved and their relation to other elements. More precisely, a feature X is not sufficient to define a class A, if there is an object that does not belong to A and has feature X. And, conversely, if a member of class A does not have feature X, then X is not necessary in the definition of A. Based on an initial draft, the authors engaged in systematic

discussions, one concept at a time, on how to better expand each concept until final agreements were reached. Most of the reviewed concepts fall under the scope of the Sequence Ontology, which aims to define sequence features used in biological sequence annotation, to which final definitions were added or updated⁶. These updated definitions are also being incorporated into RegulonDB, a database that has curated knowledge on transcriptional regulation in *E. coli*⁷ for the past three decades and populates gene regulation data in EcoCyc⁸, a scientific database for *E. coli* K-12 MG1655.

Below, we begin with a brief overview of bacterial transcription initiation. Next, we contrast classic concepts and terms relating to bacterial transcription initiation and its regulation with their current use, in light of the current body of knowledge on transcriptional regulation in *E. coli*⁷ and other bacterial organisms. We aim to construct up-to-date and precise definitions that support a logically consistent conceptualization of gene regulation. We believe this will provide a reference for knowledge representation, for modelling and for future thinking about bacterial gene regulation. Indirectly, these concepts may also influence gene regulation frameworks beyond bacteria. Throughout the manuscript, terms followed by [G] can be found in the glossary at the end of the document.

Overview of transcription initiation

The first step in transcription is the formation of the RNA polymerase (RNAP) holoenzyme ($E\sigma$)⁹, a molecular complex composed of the core RNAP plus a σ factor, which is capable of initiating gene transcription at specific DNA positions. Bacterial RNA polymerase (RNAP) is a multi-subunit enzyme. The core RNAP is comprised of five subunits: α_2 , β and β' , and ω . Core RNAP contains the active site that catalyses the formation of the phosphodiester bond of nascent RNA. RNAP α subunits interact with the upstream promoter (UP) element, which consists of two distinct subsites located upstream of the -35 element¹⁰. The RNAP core enzyme interacts in a sequence-specific manner with the template-strand positions -4 to $+2$, which constitute the core recognition element (CRE)¹¹. To form the holoenzyme $E\sigma$, RNAP is associated with a σ factor. There are two structurally and evolutionary distinct families of σ factors: σ^{54} and σ^{70} . σ^{70} -related factors contain up to four functional domains ($\sigma 1-4$). The $\sigma 2$ domain recognizes and interacts with the -10 element, and the $\sigma 4$ domain interacts with the -35 element. The extended -10 element interacts with $\sigma 3$, and this interaction is crucial in promoters whose -35 and -10 elements show a poor match to consensus sequences¹². Some promoters, particularly the ones that respond to amino acid starvation, have an element called discriminator, which is recognized by and interacts with the $\sigma 2$ domain (conserved region $\sigma 1.2$)^{13,14}. σ^{70} bound to the nontemplate strand captures the -10 region in an open complex and allows the single-strand template DNA to enter the active site. For this, σ^{70} does not need an energy source such as ATP or GTP¹⁵. σ interacts with different promoter elements to position the $E\sigma$ to unwind the double-stranded DNA in the region of the transcription start site (TSS), which corresponds to the first base of the transcript⁶. Most bacteria rely on different σ factors to take $E\sigma$ to different sets of promoters in response to changes in growth conditions¹⁶. Alternative σ factors are classified into two evolutionary distinct families: σ^{54} and σ^{70} . σ^{54} is a single member family, whereas σ^{70} normally has several members, whose number varies among bacterial species. For example, σ^{24} , σ^{28} , σ^{32} , σ^{38} and σ^{70} are members of the σ^{70} family in *E. coli*¹⁷.

$E\sigma$ initially binds promoters in a closed complex (RPC), whereby it covers DNA from approximately -55 bp to approximately $+15$ bp relative to the TSS (positive numbers represent bases downstream of the TSS, whereas negative ones represent upstream positions), according to DNA footprints¹⁸. This binding triggers a series of conformational changes both in the DNA and in $E\sigma$ that create an open complex (RPO), culminating in the separation of DNA strands from approximately positions -11 to $+3$ bp¹⁹. The region where the DNA strands are separated is often referred to as the ‘transcription bubble’, and it includes the base on the template strand, designated $+1$, that will act as the template for the first nucleotide of the transcript^{19,20}. Transcription initiates with a short unstable region potentially subject to abortive transcription, in which $E\sigma$ synthesizes short products before transitioning to a stable elongation complex²¹. The transcription cycle then proceeds with elongation and termination steps, which have been reviewed in detail elsewhere²².

The role of promoter elements is primarily to interact with $E\sigma$ to dock the DNA- $E\sigma$ complex that is competent for the subsequent steps of transcription; hence, promoter elements determine the autonomous activity of the promoter²³. The activity of different promoters varies by many orders of magnitude, ranging from promoters that produce less than one RNA copy per cell generation to promoters which generate tens of thousands of RNA copies^{24,25}.

Essentially all promoters are subject to regulation, either indirectly, by changes in $E\sigma$ concentration²⁶ or substrate concentrations^{27,28}, or directly, by specific regulators. A subclass of these regulators includes activators [**G**] and repressors [**G**], collectively known as transcription factors (TFs), that act by binding to specific DNA targets. Promoters prone to activation are often intrinsically weak owing to their low affinity for $E\sigma$, with activators compensating for this weakness by recruiting $E\sigma$ ²⁹. By contrast, repressors prevent $E\sigma$ from transcribing, often by directly occluding the promoter, or preventing some isomerization step³⁰.

The activity of most DNA-binding transcription regulators is coupled to outside signals by a variety of mechanisms that facilitate quick responses and make gene expression sensitive to environmental changes³¹. Parallel mechanisms sensitive to internal and external changes support our molecular understanding of genetic developmental programmes in eukaryotes^{32,33}.

Promoter

The original definition of promoter as defined by Jacob and Monod in 1964 is a sequence located between the operator [**G**] and the beginning of the operon [**G**] that is indispensable for operon initiation of gene expression³⁴. This definition deserves to be revised; the following discussion shows that there is no well-defined sequence that can define any promoter.

Core RNAP transcription—Although core RNAP can transcribe single-stranded DNA in nicked regions or from DNA ends, and it has been shown that RNAP can initiate transcription from double-stranded, circular DNA^{35,36}, sites of core RNAP transcription

initiation are not promoters, because they are not specific. Thus, a promoter is not necessary for random transcription, although it is essential for transcript initiation at specific TSSs³⁶.

Promoter sequence motifs—One of the most prominent characteristics of promoter sequences is the presence of $E\sigma$ recognition elements. There is a long history of promoter sequence comparisons and mutations, from which the base sequence consensus motifs [G] for -35 (Ref. ³⁷), -10 (Ref. ³⁷⁻³⁹), extended -10 (Refs. ^{12,40}), -12 and -24 (Ref. ⁴¹), discriminator⁴², the Core Recognition Element (CRE)¹¹, and the Upstream Promoter (UP)^{10,43} elements were identified. Instances of these motifs interact with the σ and α subunits of RNAP.

A variety of old and recent evidence points to the existence of sequences that conform to the motifs but does not support transcriptional activity, such as the presence of promoter-like sequences involved in transcriptional pausing [G]⁴⁴, the presence of a high density of σ^{70} promoter-like sequences in intragenic regions⁴⁵⁻⁴⁷ and, in some bacteria, the overrepresentation of the -10 element in coding sequences⁴⁸. Thus, the presence of motifs is not a sufficient condition for promoter activity.

Furthermore, the presence of a sequence in a DNA segment matching a motif is not necessary to regard a segment as a promoter. It is known that weak promoters may lack one or several functional elements, which can be compensated for by activators²⁹. Furthermore, there may be sites that have poor matches to motifs but are recognized efficiently by $E\sigma$; it has been shown that a single mutation over random 100 bp DNA stretches can generate a promoter^{49,50}.

$E\sigma$ binding does not define a promoter— $E\sigma$ binding sites that drive transcription are defined as promoters. For both families of σ factors, there is evidence that $E\sigma$ binding is not sufficient to define a promoter. For example, there are sequences that conform to the motifs but do not support transcriptional activity, such as sequences leading to unproductive binding of $E\sigma^{70}$ (Ref. ⁵¹). Similarly, it has been suggested that $E\sigma^{70}$ binds in an inactive conformation under salicylic acid stress, because it was observed bound adjacent to strongly downregulated genes⁵².

$E\sigma^{54}$ can bind promoters in a transcriptionally inactive state, and its activation requires an enhancer-binding protein (EBP)⁵³. Genome-wide studies have greatly increased the number of known $E\sigma^{54}$ binding sites in *E. coli*^{54,55} and other bacteria^{56,57}. Many of the newly found sites are intragenic, and most of them are not conserved in other species; thus, not all of them are likely to be functional. Hence, the binding of $E\sigma$ to a site is not a sufficient criterion to regard those sites as promoters (Fig. 1a).

Some promoters, comprising elements that are very different from the consensus sequence or that lack some recognition element, cannot bind $E\sigma$ alone; they require additional factors for their function. For example, the λ phage PRE promoter has -10 and -35 elements that differ from the consensus sequence, and it requires cII protein for $E\sigma$ binding¹². Therefore, autonomous binding of $E\sigma$ to a sequence, that is, without the need for other molecules, is not a prerequisite for a sequence to be a promoter (Fig. 1b).

Although transcription initiation by $E\sigma$ may not lead to a functional RNA, it nevertheless requires a promoter. Some promoters generate short non-functional transcripts 2–15 bp long as a result of abortive transcription; this phenomenon plays a role in the regulation of transcription⁵⁸. Non-functional RNAs result also from so-called TSS-associated RNAs of around 35–50 bp⁵⁹ and other pervasively transcribed spurious RNAs^{60,61}.

Regulatory binding sites are not part of the promoter—Some $E\sigma$ binding sites require the binding of activators to initiate transcription^{12,62}, which raises the question of whether to annotate both the $E\sigma$ binding site and the required activator sites as part of the promoter. We propose not to do so, because not all promoters are activator-dependent, and it is thus not a necessary feature. In cases where the activator site overlaps the promoter, the annotated promoter will include the activator site; currently, RegulonDB contains at least 40 activator sites of 22 different TFs that overlap their corresponding promoter. However, the overlapped sequence should be annotated again independently as a regulatory site (discussed below). In cases where the activator site does not overlap the promoter, the region annotated as promoter should not include the activator site. Although this promoter sequence is not competent for transcription on its own, being annotated as a promoter indicates that it is the sequence recognized by RNAP, with the help of the activator to initiate transcription. Similarly, repressor sites and overlapping promoters are separate entities, since they are not necessary for promoter activity, although they are necessary for regulation. The existence of overlapping elements in DNA is a recurring theme in the modelling of transcriptional regulation.

Different σ factors initiating at the same TSS define different promoters—Overlapping promoters can initiate transcripts at the same TSS^{63,64}. For example, *glmY* expression in *E. coli* is controlled by two overlapping promoters, one recognized by σ^{54} and the other by σ^{70} (Ref. ⁶⁴). 5'-Rapid Amplification of cDNA Ends [G] (RACE) analyses showed that these promoters initiate transcription of the *glmY* gene at the same position. Mutations in the -10 element, recognized by σ^{70} , and in the -24 element, recognized by σ^{54} , abolished the corresponding $E\sigma$ activity while leaving the activity of the second $E\sigma$ unaffected⁶⁴. This finding demonstrates that RNAP holoenzyme is using different recognition elements even when selecting the same base as TSS. Similarly, $E\sigma^{70}$ can initiate transcription from the majority of σ^{32} promoters at identical TSSs, and $E\sigma^{70}$ transcribes 40% of σ^{24} promoters⁶⁵.

Fig. 2 shows the numbers of TSSs targeted by different σ factors, according to the information in RegulonDB⁷. We propose that overlapping binding sites targeted by different σ factors be considered separate promoters, even if the TSSs are the same. Certainly, such cases may be subject to different regulatory inputs, and different sequence elements will be used, according to the nature of the σ factor.

Proposed definition for promoters—In summary, promoters are essential for transcription that is specific; an instance of a sequence motif is not mandatory; autonomous binding of $E\sigma$ is neither sufficient nor necessary; and promoters are σ factor-specific. Based on these considerations we define a promoter as a DNA segment essential for the specific initiation of transcription at a defined location in a DNA molecule, although this location

might not be one single base. It is recognized by a specific $E\sigma$, and this recognition is not necessarily autonomous.

Transcription factors

In the operon model, the product of a regulator gene, the cytoplasmic repressor, acts on the operator to affect the synthesis of a set of genes^{66,67}. The chemical identity and mechanism of repressors were unknown until the *lac* and the λ phage repressors, proteins with high specificity to a site in the DNA, were isolated^{68,69}. Later, an $E\sigma$ binding site was found to overlap λ repressor and *lac* repressor operator sites, which confirmed the mechanism for a repressor, which prevents RNAP from binding to the promoter⁷⁰. It was assumed that gene regulation was mediated solely by repressors, until genes in the maltose and arabinose operons and in λ phage were found to be positively regulated^{71–73}. Now, activators and repressors are collectively called TFs, and their sites of action are called transcription factor binding sites (TFBSs).

Many factors affect transcription—The term TF should not be confused with the more general class of factors that regulate transcription. Regulators that act on RNA, DNA or RNAP throughout the whole transcription cycle, including proteins, small peptides, noncoding RNAs and a variety of small ligands, have also been referred to as TFs^{20,74}.

Originally, regulators of transcription initiation were thought to be proteins exclusively; however, there are other kinds of molecules that regulate transcription initiation, such as regulatory RNAs^{75,76} and small ligands, for example, ppGpp⁷⁷. Here, we deal only with regulatory gene products as originally conceived by Jacob and Monod. For instance, *E. coli* 6S RNA regulates transcription initiation by directly binding to $E\sigma$ ⁷⁰ and preventing its binding to the promoter, leading to an increase in $E\sigma$ ³⁸ transcription^{78,79}. However, the term TF has a traditionally well-established meaning in all domains of life: a protein that binds DNA to regulate transcription initiation^{80–85}.

A large number of protein factors that bind directly to $E\sigma$ to regulate transcription initiation have been identified over the past 20 years^{86–89}. Moreover, some proteins that regulate transcription initiation bind to both DNA and $E\sigma$. Thus, the criterion of mere binding to a molecule, be it DNA or the holoenzyme, makes the definition of transcription factor imprecise.

By contrast, specificity — the ability to promote or repress the expression of a subset of genes — must be a feature of any regulator of gene expression, as it is the means to differentially respond to different conditions. This criterion can be used to decide whether a regulator that binds both the DNA and any component of the $E\sigma$ is a TF. If the specificity of the regulator is determined directly by the sequence of the DNA to which it binds, then it is a TF. If it is determined by its interaction with $E\sigma$, then we propose to use the term $E\sigma$ -centered regulatory protein⁹⁰.

To refine the TF definition, we focus on proteins that specifically bind to DNA and regulate transcription and asked if they should be covered by this term.

σ factors—Both TFs and σ factors bind DNA and lead $E\sigma$ to different sets of promoters in response to different environmental conditions. For example, the synthesis of σ^{29} is induced during sporulation in *Bacillus subtilis*, thereby enabling the transcription of the subset of genes required during sporulation⁹¹. Another example is *E. coli* σ^{32} , whose expression is induced under heat-shock conditions; in turn, $E\sigma^{32}$ induces the expression of the heat-shock response genes^{92,93}. σ factors could be regarded as activators, as they were initially discovered as factors that increase transcription activity *in vitro*⁹⁴. However, σ factors can be defined as the proteins that regulate and are essential for specific transcription initiation while being part of the RNAP holoenzyme⁹⁵. The features that make σ factors different from TFs are the ability to confer core RNAP promoter specificity, open duplex DNA and facilitate template strand entry into the RNAP active site⁹⁶.

Some TFs have activities that are very similar to those of σ factors, such as forming a complex with RNAP in solution or stabilizing the open complex (RPO). For example, in *E. coli*, SoxS forms a complex with $E\sigma$, which then scans DNA using SoxS to search for their cognate sites, called Sox boxes^{97,98}. Although sequence specificity of SoxS has been demonstrated⁹⁹, it does not aid in DNA opening and template strand entry to the active site; instead, this TF acts by pre-recruitment of $E\sigma$. CarD and RbpA proteins bind both $E\sigma$ and the promoter just upstream of the -10 element to stabilize the unstable open complex (RPO) in *Mycobacterium sp.*^{100–102}. Since specificity of CarD and RbpA have not yet been shown to be determined by the DNA sequence, these are RNAP-centred regulatory proteins⁹⁰ that help σ to stabilize the open complex.

Nucleoid-associated proteins—The distinction between TFs and nucleoid-associated proteins (NAPs) is a perfect example of how a preconceived idea of two different types of molecules, based on genetics and function, led to the realization that TF and NAP functions are frequently performed by the same molecules¹⁰³. NAPs are a group of DNA-binding proteins that are believed to play important roles in chromosome structure and compaction^{104,105}. Some NAPs have been shown to function as site-specific regulators of transcription initiation^{104,106–114}, similarly to other TFs, as well as acting at a distance from the target promoter by bending the intermediate DNA; integration host factor (IHF) is a well-known example at σ^{54} promoters^{115,116}. Some NAPs tend to bind to many sites with fairly low sequence specificity¹¹⁷, and most global regulators [**G**] in *E. coli* are NAPs^{103,118}. As NAPs have functions that overlap with those of TFs, for the purposes of the definitions we propose we do not consider them as a separate class.

Proposed definitions for TFs—A comprehensive terminology to describe all types of regulatory gene products that act on the different levels of transcription and/or other mechanisms of gene regulation is beyond the scope of this article; however, we outline terminology to designate different kinds of regulators. In the words of Jacob and Monod, we can begin by defining the general term ‘regulatory gene product’ as any gene product that increases or decreases the expression of a specific set of genes (note that gene product complexes such as heteromultimeric TFs are included, for example, IHF in *E. coli*).

We can distinguish two general kinds of regulatory gene products: regulatory RNA and regulatory proteins. These we can further subclassify according to the level at which they act

to regulate gene expression or the specific mechanism of regulation. In particular, we have to define the class ‘DNA-binding regulatory protein of transcription initiation’ as the subclass of regulatory proteins that bind to specific DNA sequences to regulate transcription initiation. This class includes TFs, that is, DNA-binding regulatory proteins that bind near a promoter and affect transcript initiation at that promoter, and sigma factors, that is, DNA-binding regulatory proteins of transcription initiation that are part of the RNAP holoenzyme and are essential for specific initiation of transcription. Another subclass of regulatory proteins would be ‘RNAP-centred regulatory protein of transcription initiation’, defined as the proteins that regulate transcription initiation by interacting with $E\sigma$, and whose specificity is not determined directly by recognition of specific DNA sequences.

Transcription factor binding sites

TFs bind specifically to their binding sites to activate or repress adjacent promoters. Recent genome-scale technologies capable of identifying TFBSs anywhere in the genome, such as chromatin immunoprecipitation followed by sequencing (ChIP-seq) or by identification using a DNA array, or chip (ChIP-chip) have shown that sites where TF binding has no direct effect on transcriptional regulation are common (Fig. 3). For instance, when analysing 12 studies of 9 different TFs in *E. coli*, only ~25% of 3,973 TF-gene interactions showed evidence of regulating local gene expression¹¹⁹. Similar observations have been made in other bacterial genomes, such as *Mycobacterium tuberculosis*^{4,120}, *Pseudomonas aeruginosa*^{121,122}, *Salmonella enterica*¹²³, *Listeria monocytogenes*¹²⁴, *Helicobacter pylori*¹²⁵ and *Shigella flexneri*¹²⁶. The diverse behaviour of TFs at the genomic level raises the question: which fraction of TFs has binding sites that are exclusively involved in transcriptional regulation and thus behaves like MelR¹²⁷, OmpR¹²⁸ and LexA¹²⁹? Indeed, most TFs have sites that support other functions, such as contributing to the nucleoid structure of the genome, akin to NAPs¹³⁰.

It will be interesting to understand the different distribution of TFBSs in noncoding versus coding regions. For instance, two-thirds of 96 binding sites of the regulator of iron homeostasis Fur lie in intergenic regions¹³¹. Moreover, a genome-wide study of uncharacterized TFs in *E. coli* identified binding sites for 10 candidate TFs using ChIP-seq combined with exonuclease treatment (ChIP-exo) and showed that only 41% of 241 sites were in regulatory regions¹³². By contrast, as an extreme case, 70% of binding sites for RutR were found within coding regions in *E. coli*, a tendency conserved in other bacteria¹³³.

Additionally, a more clear distinction of subclasses of binding sites is emerging, either owing to the contribution of additional TFs^{129,131,134} or the same TF working differently in varying conditions. For example, Fur was shown by ChIP-seq to bind to lower and higher numbers of TFBS under aerobic or anaerobic conditions, respectively¹³¹. In another study, ChIP-seq combined with RNA sequencing (RNA-seq) under nine physiological conditions showed that nutrient levels or growth phase affect the mechanism of action of the TF Lrp¹³⁵.

Taken together, these studies distinguish between sites that affect transcription from those that do not. We thus propose the term TFBS to be defined as a DNA site where a TF binds specifically and that the term transcription factor regulatory sites (TFRS) be defined as the subset of TFBSs that are involved in transcription regulation (Fig. 3).

Architecture of regulatory regions—TFRSs, initially termed operator sites, were conceived as a single entity located near a cognate regulated promoter^{34,136}. However, only 37% of all current promoters in RegulonDB are regulated by a single TFRS. Certainly, the steady increase of well characterized regulated promoters has led to what we now see as the rich architecture of regulatory regions, with promoters subject to the effect of one or several TFs binding to one or several TFRSs.

The diversity of TFRSs close to promoters became clear as a result of studies in the early 1990s in an initial review of 107 σ^{70} and 12 σ^{54} -dependent regulated promoters³. A general principle emerging from this cohort of bacterial regulatory regions was the requirement for a proximal site, defined as an activator or repressor site located in positions that enable direct interaction of the TF in $E\sigma^{70}$ promoters. Only 4 out of 107 σ^{70} promoters lacked a proximal site^{3,137}, a finding that has been sustained as more promoters have been characterized¹³⁸. In one study, only 6.9% of σ^{70} promoters (48 out of 692) lacked a proximal site located between -95 to +20 relative to the TSS⁷. Note that promoters were counted individually even if multiple promoters coexisted in the same upstream region.

This principle does not apply to σ^{54} -dependent promoters, the activator sites of which are more distal, with the activator often brought close to the promoter by DNA looping induced by IHF binding between the enhancer-like TF sites and the promoter^{139,140}. This different organization is associated with the capability of $E\sigma^{54}$ to form stable but inactive closed complexes and the absolute requirement of an activator for transcript initiation, as opposed to the $E\sigma^{70}$ holoenzyme, which is competent to initiate transcription without activators and forms a transient closed complex^{3,141}.

Regulatory modules or phrases—The collection of sites affecting a promoter can be partitioned into groups of sites that work together, similar to words in a sentence that form syntactic categories. These ‘regulatory phrases’ or ‘*cis*-modules’¹⁴² can be homotypic modules, grouping sites for the same TF, or heterotypic modules, grouping sites for different TFs that work jointly in the regulation of a promoter (Fig. 4). These modules constitute the building blocks of grammatical¹⁴³, combinatorial logic^{143,144} and of quantitative thermodynamic models of regulated promoter transcriptional activity^{145–147}. They are inferred in approaches searching to understand large amounts of gene expression data¹⁴⁸ and aim at a combinatorial construction of all possible regulatory arrangements in bacterial genomics. For instance, a grammatical model was implemented with a reduced number of rules to generate the whole collection of regulatory architectures of σ^{70} -regulated promoters¹⁴³.

We propose to define a bacterial TFRS module, or TFRS phrase, as a combination of one or several TFRSs whose bound TFs work jointly in the regulation of a promoter. A bacterial TFRS collection is defined as all the TFRSs that regulate a promoter.

Operon

The classic definition of an operon is the units of coordinated expression constituted by an operator and the group of structural genes coordinated by it¹³⁶, thus requiring an operon to have one operator. However, multiple TFRSs organized in a module can regulate a single

promoter. Furthermore, transcriptional regulation of some co-transcribed genes may be independent of TFs. For example, the genome of *Mycoplasma genitalium* encodes a limited number of TFs, and it has been suggested that this bacterium depends mostly on DNA supercoiling [G] for gene expression regulation^{149,150}. For example, the –10 element along with DNA supercoiling induced by hyperosmolar conditions have been shown to be sufficient for the induction of expression of the *MG_149* gene¹⁵¹. Because some cotranscribed genes may not be regulated by TFs, we propose TFRSs to be considered independent of these ‘units of coordinated expression’ and their connections captured by defining their regulatory relationships.

Cotranscribed genes are not limited to one pathway—The first operons studied coordinate the expression of genes whose products are involved in a common pathway (that is, lactose, maltose or arabinose). Hence, it was reasonable to expect that co-transcribed genes of an operon participate in the same biological pathway. However, not long after proposal of the operon model, an early example of co-transcribed genes involved in different pathways was found; in *Bacillus subtilis*, the tryptophan gene cluster is expressed coordinately with genes involved in histidine and tyrosine production under tryptophan-limiting conditions¹⁵². Comprehensive studies of functional classes of large numbers of transcripts in *E. coli* now provide us with numbers that underscore the diversity of functions of many co-transcribed genes¹⁵³.

Although the operon concept was initially proposed to account for the discovery that units of transcriptional regulation in bacteria were not single genes, it was later extended to include monocistronic operons [G] by Jacob and Monod themselves^{154,155}. Thus, we consider it to be unnecessary for an operon to have more than one gene.

Transcription units versus operons—The most general definition for both operon and transcription unit is a set of adjacent co-transcribed genes (Supplementary table 1), although there are ambiguities between the two concepts. We think that it is better to define transcription units as physical entities, whereas operons are more complex conceptual entities. At least in theory, every transcription unit in a cell can be determined by measurement (for example, cDNA sequencing). In the literature, there are two ways to define transcription units: they are segments of DNA that extend from the promoter to the terminator^{156,157}, which are included, or they are DNA segments that begin at a TSS and end at a transcription termination site (TTS)^{158,159} (Supplementary table 1). The latter, and most common usage, is equivalent to defining transcription units as the DNA that corresponds to a primary transcript.

A promoter can have more than one TSS, and a terminator can have more than one TTS. Promoters were found to have on average 1.6 TSSs in an integrated genome-wide analysis of *E. coli*¹⁶⁰. If we opt to define transcription units as the DNA sequences that begin at a TSS and end at a TTS, we would be representing multiple units that differ by a few nucleotides, but most of this microvariation can be considered functionally spurious. Studies using single-molecule fluorescence resonance energy transfer (FRET) supported a model in which this microvariation arises from the thermodynamics of transcription⁵. Thus, we prefer to

think of these units of transcription as having variable ends and only consider the predominant ones for each promoter or terminator (Fig. 5a).

Nonetheless, some regulatory mechanisms alter TSS selection from a single promoter. For example, the TSSs of the *pyrC* and *pyrD* promoters of *E. coli* and *Salmonella enterica* are shifted by nucleotide concentration^{27,161}. *pyrC* encodes a pyrimidine biosynthetic enzyme in *E. coli* and *S. enterica* serovar Typhimurium. Transcription of *pyrC* initiates at four adjacent sites named T6, C7, C8 and G9, each of which produces a different transcript. Under conditions of pyrimidine excess, the intracellular level of CTP is high, and position C7 is the dominant start site^{28,161}. C7 transcripts are not translated, because they form a stable hairpin at their 5' ends that blocks ribosome binding to the *pyrC* Shine-Dalgarno (SD) sequence. Under conditions of pyrimidine limitation, the GTP level is high, which makes the G9 start site dominant. G9 transcripts do not form the inhibitory hairpin and are readily translated²⁸. Thus, C7 and G9 are different non-spurious TSSs from the same promoter (*pyrCp*), because they originate two transcripts that are differentially translated. Thus, these are two transcription units originating from the same promoter²⁸.

In addition, some promoters can be primed by nanoRNAs in a growth phase-dependent manner, thereby altering TSS selection¹⁶². Regarding TSSs, it has long been known that Rho-dependent termination is diffuse, and elongation factors can conditionally allow bypass of terminators, which results in multiple termination points^{74,163–165} and complex patterns of expression^{166–168}. To include microvariation subject to differential regulation, we propose to define transcription units as DNA regions delimited by different nonspurious TSS–TTS pairs.

A widely held distinction between transcription units and operons is that one gene can belong to one or more transcription unit but only to one operon. This distinction comes from the existence of promoters and terminators internal to operons. In 1967, dis-coordinated expression of the cluster of five tryptophan synthesis genes of *Salmonella typhimurium* was reported¹⁶⁹. Deletions ranging from the operator to the second gene of the cluster suppressed expression of the first two genes only. The last three were silenced after the deletion reached the region between the second and third genes. Deletion of the operator upstream of the five genes deregulated all of them. These five genes were considered an operon regulated by a single operator but subdivided into two parts determined by promoter-like elements. Similarly, the *glnALG* operon is differentially transcribed in different transcription units depending on the nitrogen source by means of an internal terminator and alternative promoters¹⁷⁰. Now, many systems in which subsets of genes of an operon are differentially expressed under different conditions due to alternative combinations of promoters and terminators are known^{158,160,168,171–175}. This has led to the notion of operons with internal promoters and terminators as containing several transcription units.

However, internal promoters are often differentially regulated. Currently, 862 operons with known regulation are listed in RegulonDB. Of these, 143 operons consist of more than one transcription unit. Of the 143 multi-transcription unit operons, 92 are differentially regulated, whereby at least one pair of their constituent transcription units is regulated by different TFRS collections. Moreover, there are cases in which the genes of an operon are

not all co-transcribed. This phenomenon is generally described as an operon containing genes ABC with two transcription units, AB and BC (Fig. 5b).

To preserve both the notion of operons being the set of genes in the same transcription unit and the notion of the set of genes coordinated in the maximal set of overlapping transcription units, we propose to use the term simple operon for the former, and complex operon for the latter. Although some complex operons may have genes that are not co-transcribed, there is ‘co-operation’ of their function (they use the same infrastructure); despite being differentially regulated, there is likely a complex functional interplay among the individual transcription units that makes it hard to consider them individually.

Whereas operons are defined in terms of genes, we think that transcription units do not necessarily bear genes or have a function. Transcriptome analysis has revealed the widespread production of non-canonical transcripts^{60,61}, that is, transcripts that are noncoding and are often antisense; such ‘pervasive transcripts’ rarely have an assigned function^{176,177}. Evolutionarily, we should not assume that transcriptional regulation has been selected as ‘optimal’, that is, to express exactly the right genes at the right moment in the right cells^{178–180}. Non-functional transcripts may constitute raw material for evolution given novel mutations. Moreover, pervasive transcription may have a function in itself, that is, a basal level of pervasive transcription means that core RNAP or σ levels have to be higher, effectively buffering their levels in the cell¹⁸¹.

Co-expression may extend beyond operon limits. Significant coexpression has been observed in regions of 10 kb and even larger regions, which has been associated with transcriptional read-through [G], supercoiling and nearby regulons^{166,167,182}. Read-through of terminators has been documented^{183,184}, and a recent transcriptome obtained using single-molecule long-read sequencing has extended 34% of RegulonDB operons by at least one additional gene¹⁶⁸.

Proposed definitions for operons—An operon is a set of adjacent genes whose transcription is coordinated by one or several mutually overlapping transcription units that are transcribed in the same direction and share at least one gene. A simple operon is an operon whose transcription is coordinated from a single transcription unit. A complex operon is an operon whose transcription is coordinated through several mutually overlapping transcription units that are transcribed in the same direction and share at least one gene.

Regulon

A regulon is a system in which the production of all enzymes (of a metabolic pathway) can be controlled by a single repressor substance; this substance may consist of several entities, but whatever its nature, it acts in a unitary fashion¹⁸⁵.

Although regulons were originally proposed by studying the pathway of arginine biosynthesis, they must now be defined exclusively by regulation. From the 149 described *E. coli* regulons that include enzymes catalysing metabolic reactions, only 21% included enzymes for a single metabolic pathway whose inputs and outputs form a connected network¹⁸⁶.

What kind of regulatory entity defines a regulon?—We suggest that the regulator that defines a regulon must be a regulatory gene product. Before this discussion, Sequence Ontology used to refer to the regulator as “regulatory signal”⁶. However, the term “signal” is used to refer to cues that are transformed into an effector that interacts with regulators, thereby providing information about environmental and physiological states so that the cell can adjust gene expression levels (see below). Although most TFs bind to one effector, there are documented cases where TFs allosterically bind several metabolites, for example, tryptophan, tyrosine and phenylalanine in the case of TyrR¹⁸⁷. Conversely, small molecules may act as effectors for more than one TF, such as Zn⁺² binding to ZntR and Zur, and tryptophan to TyrR and TrpR. Thus, regulation by an effector does not imply regulation by a specific TF. The initial Sequence Ontology definition of regulons corresponded to that of stimulons [G]¹⁸⁸.

Based on the original definition, a regulator entity should be either one regulator that can work independently (with one or multiple TFRSs) or any collection of regulators working in unity, such as complex heteromultimeric proteins. Currently, RegulonDB has documented 598 transcription units regulated by more than one TFRS; of these, 477 are regulated by different TFs. These data motivate the expansion of a regulon to include groups of genes subject to multiple regulators. They will not be acting in unity but will support a complex multiple input–output regulation. Such sets of multiple regulators may be, for instance, TFs comprising TFRS-modules anchored by a proximal site, or alternatively, the set of TFs binding the TFRS collection (Fig. 4). How these groupings will help to map mechanisms to physiology is an open question.

Regulated entity and regulated stage of gene expression—Regulator, regulated entity and the level at which gene expression is regulated are interdependent features. The concentration of products of genes transcriptionally coordinated may be uneven¹⁸⁹, implying that the mapping of transcription units to translation units is complex. Most regulatory RNAs regulate gene expression post-transcriptionally by base pairing with mRNA. Thus, if the regulon definition is such that it includes RNAs as regulator entities, then regulated entities should include transcription units and transcribed coding sequences.

Proposed definition for regulons—In general, units of gene expression are defined as transcription units or transcribed coding sequences. A regulon is a set of units of gene expression directly regulated by a common set of one or more common regulatory gene products. A simple regulon is a regulon defined by considering one regulatory gene product, and a complex regulon is a regulon defined by considering the units of expression regulated by a specified set of regulatory gene products.

Signal and effector

Effectors were originally defined as compounds that bind specifically and reversibly to an allosteric site on a protein and bring about a discrete reversible alteration of its molecular structure that modifies its properties, changing one or several of its kinetic parameters^{154,190}.

It is more difficult to trace the classic definition of a signal. As we want to define the term signal in the context of gene regulation, one appropriate definition to consider is a molecule

originated from the environment or produced by metabolism to which a cellular response must be mounted¹⁹¹.

Difference between signal and effector—The concept of signal operates at the physiological level, whereas the effector is critical at the mechanistic level of gene expression. The signal is the starting point of an information flux that will use a variety of reactions and mechanisms ultimately reaching the regulatory machinery of cells. Effectors provide the necessary continuity to information flux by binding to the regulator that modifies gene expression (Fig. 6). Originally, effectors modulated protein activity. We propose to generalize the definition of targets of effectors to include all kinds of regulatory gene products. Some mRNAs have motifs to which small molecules bind to modify secondary structure, thereby regulating gene expression¹⁹².

In this flux, an effector action has been considered a reversible allosteric transition that changes some chemical (kinetic or affinity) parameter of a regulatory molecule¹⁵⁴. The effector concept must now be extended. Reversibility and allosteric features are no longer necessary, some effector-induced changes result in proteolysis, such as for *E. coli* LexA^{193,194}, and covalently attached groups that irreversibly change TFs are also considered a chemical change produced by effectors. Phosphotriester and 6-*O*-Methylguanine DNA lesions irreversibly methylate Cys residues of Ada protein, a TF that induces its own expression¹⁹⁵. Furthermore, the activation of this TF is not due to a conformational change but to changes in the electrostatic repulsion between the protein and the DNA¹⁹⁶.

Signal and effector are not disjointed concepts. An example that fits perfectly with the 1963 definition of effector is that of an extracellular molecule that binds to a transmembrane sensor that reversibly modifies its conformation. This effector also acts as a signal, because the conformational change of the transmembrane sensor triggers an intracellular cascade of protein–protein interactions, that is, the signal transduction pathway, that ends in the activation or inactivation of a TF, which will in turn repress or activate the transcription of its target genes (Fig. 6).

What kinds of entities are signals and which are effectors?—Some signals are not material in nature. Environmental changes are mostly complex and elicit a plethora of changes in metabolic fluxes that generate internal signals in the form of changes in concentration and ratios of metabolites¹⁹⁷.

We propose to extend Ptashne's view, that any kind of molecule can be an effector³³, to include other kinds of entities such as temperature and light, as they can induce conformational changes in DNA, in proteins and in RNA, thereby regulating gene expression. It has been proposed that changing from a cooler environment to a warm host triggers virulence factors in bacteria^{198–200}. For example, in *Salmonella typhimurium*, high temperature unfolds the autorepressor TlpA, preventing dimerization^{201,202}. TlpA monomers are unable to bind DNA. High temperature can directly melt mRNA-inhibitory structures that prevent binding of the ribosome, thereby inducing translation initiation^{201,202}. Since RNA melting is a conformational change, temperature can play the role of effector (for example, in the induction of the heat-shock σ factors *rpoH* of *E. coli* and *prfA* of

*Listeria monocytogenes*¹⁹²). An example of a transcriptional regulator that is activated by light is the antirepressor AppA of *Rhodobacter sphaeroides*. Light sensed through the BLUF domain of AppA causes the reduction of affinity for DNA of the complex it forms with the repressor PpsR, thereby regulating expression of photosynthetic genes^{203,204}.

To take into account non-material signals and effectors, we make use of the conceptualization of the Basic Formal Ontology (BFO), an upper-level domain-independent ontology that describes the most general classes under which domain-specific classes can be located. The BFO partitions reality into two general classes: continuants and occurrents. A continuant is defined as an entity that persists, endures or continues through time while maintaining its identity. It was defined in opposition to processes, or occurrents, which are entities that unfold in time²⁰⁵.

Proposed definitions for signals and effectors—Signals and effectors are any kind of continuant, where a signal is defined as a continuant that is the first step in a flow of information that causes a change in gene expression. An effector is defined as a continuant that produces a chemical change in a molecule and modifies its activity and/or specificity. More precisely, we propose the definition of an effector of gene expression regulator as an effector that acts on a regulatory component of a genetic switch.

Conclusions

Terms that historically made sense face limitations with new discoveries. As a consequence, ambiguities in their use emerge, requiring concepts to be revised and refined as natural science progresses. Problems arise when attempting to define classes of objects that include all and only the intended objects, given the abundance at all levels of unusual cases. Here, we analysed the adequacy of properties used to define elements involved in bacterial transcription initiation, considering possible extreme cases and generalizing definitions accordingly. We envision that an additional strategy to resolve ambiguities is to define terms that capture the object's most general behaviour and to allow any member of a class to have a different role in specific circumstances. For example, RNAP can have a repressor role at a convergent adjacent promoter²⁰⁶.

Original concepts regarding sequence features were defined in genetic or physiological terms. The discovery of a functional sequence normally was followed by its characterization. However, sequence motifs cannot ensure the identity of a function, and not all functional sequences are motif compliant. Motifs are not obligatory elements in the new definitions.

Deriving universally robust functional definitions of sequences is complicated by the fact that evolution in bacteria happens rapidly, and many of the revealed features might serve no biological purpose. It is likely that some features are just pawns in the evolution game, as evidenced by the fact that nearly all DNA segments of a bacterial genome can be transcribed^{61,176,177,207}. In fact, we suggest that the arrangement of DNA elements and regulatory architectures in a bacterial genome derive from the functional and anatomical properties of RNAPs, TFs and NAPs which are more conserved in evolution than DNA regulatory sequences. After analysing the different types of concepts, we can grasp some

common themes. The distinction of functional versus spurious sequences is suggested as a guiding principle to define the level of signal versus noise at which sequences are to be classed as a functional element and which are not. Thus, promoters can be defined if their activity can be measured even if they transcribe a non-functional transcript. Similarly, when defining elements, a guiding principle to consider two entities as separate is whenever they are subject to different regulation. We have followed this guidance to describe variable TSSs or TTSs as one element, and to prefer modelling the same TSS transcribed by different sigma factors as different promoters. The same is true for the same binding site sequence being recognized by two different TFs. Certainly, differential regulation has to be captured in any model of gene regulation.

A recurrent theme is that overlapping of different DNA elements does not imply that these elements are a single entity. For example, different promoters can overlap and use the same TSS but use different sigma factors; similarly, activator and repressor sites can overlap a promoter region. In this sense, the correspondence between DNA and concepts is not a one-to-one relationship.

Overall, we have expanded definitions while trying to retain their essence. But, we are aware that different generalizations are feasible; for instance, we here focused on transcriptional regulators that are gene products, but they could be expanded to include small ligands such as ppGpp and consider the ppGpp regulon²⁰⁸.

The challenge in biology is that, experimentally, we may understand a few cases. However, evolution enables a much larger combination of possibilities. Ideally, a known corpus of well-known cases will generate principles that predict the universe of all possible combinations. For example, we can think of TFRS modules anchored at a proximal site in σ^{70} -dependent promoters^{3,137} as an initial working hypothesis that restricts the range of possible promoter architectures that can be validated, or corrected, by bioengineering and synthetic approaches²⁰⁹. The challenge is to implement methods and strategies that test the validity of our current definitions on the one hand, and advance our quantitative and qualitative integrated understanding of microbial gene regulation on the other.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Operon

A set of adjacent cotranscribed genes

Activators

Gene products that increase transcription, indicating their function is to enhance promoter activity

Repressors

Gene products that decrease transcription, indicating their function is to hamper promoter activity

Operator

A genetic entity adjacent to a group of genes that regulate their expression and that is sensitive to a repressor

Motifs

Representations of a collection of binding sites that summarize their characteristics

5'-Rapid Amplification of cDNA Ends

(5'-RACE) is a method that amplifies mRNA between a defined internal site and its initiation site

DNA supercoiling

The writhe of DNA over the double-stranded axis

Monocistronic operons

Operons that encode a single gene product

Transcriptional read-through

Transcription that allows RNA polymerase to continue transcription beyond termination sites

Stimulons

Sets of genes (or sets of regulons) whose products are increased in response to a common environmental stimulus

Global regulators

TFs that affect a large number of genes involved in many different functions

Transcriptional pausing

A process in which the RNAP slows down transcription during elongation

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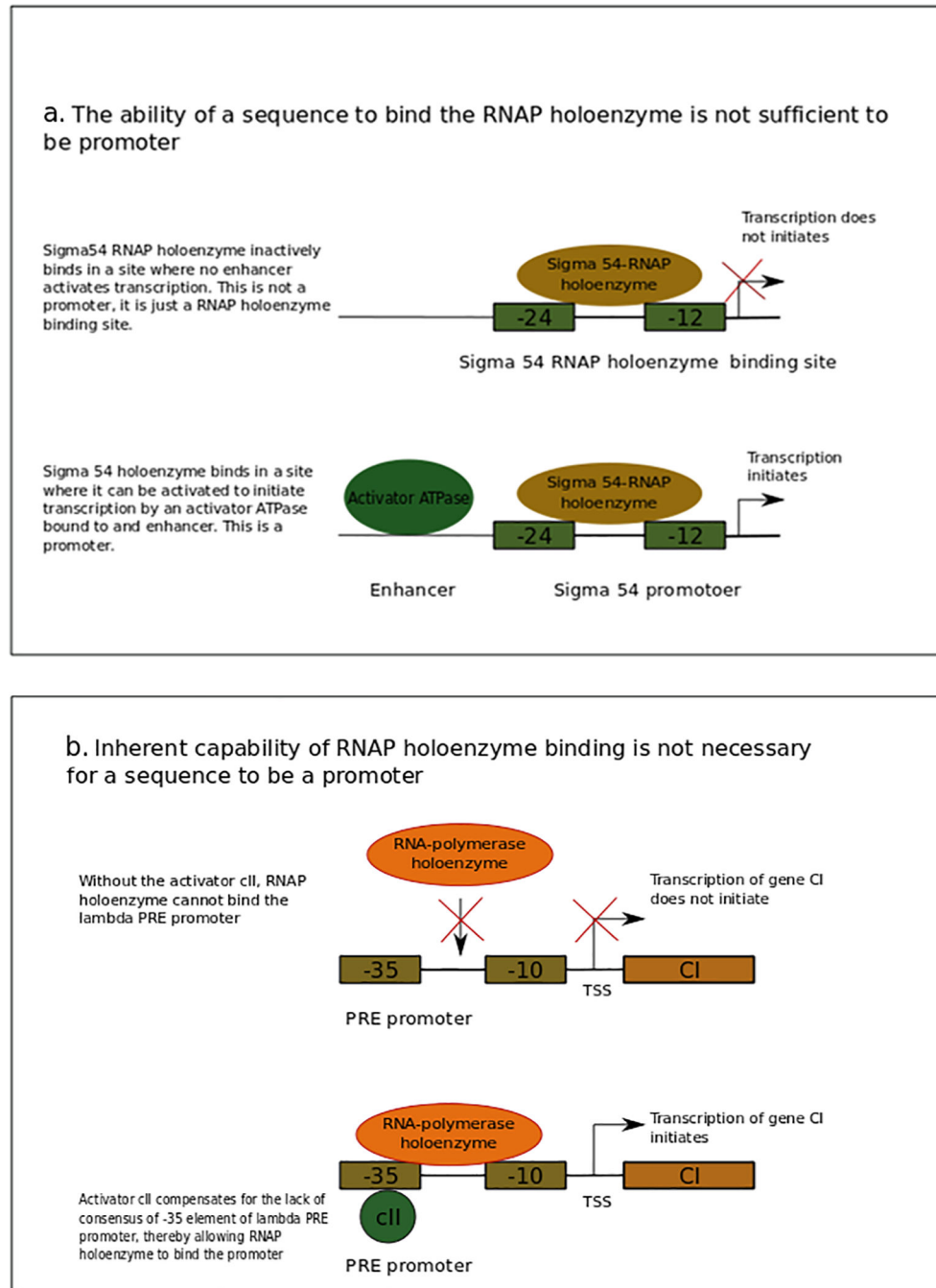


Figure 1 | RNAP holoenzyme ($E\sigma$) intrinsic recognition by a sequence is neither necessary nor sufficient for that sequence to be a promoter.

a | σ^{54} is divided into three conserved regions. Region I (not shown) comprises a domain that inhibits polymerase isomerization and initiation in the absence of activation and stimulates initiation in response to activation. Due to this inhibiting element, σ^{54} requires an ATP-dependent activator (green oval)²¹¹. Region II (not shown) is variable and is implicated in DNA melting. Region III (brown oval) is the primary DNA-binding region and recognizes the -12 element and -24 element, to which an inactive $E\sigma^{54}$ (not shown) binds.

Transcription initiates upon binding of an ATP-dependent activator to a sequence called enhancer. Binding sites of $E\sigma^{54}$ from which no transcription occurs, possibly because there is not an enhancer nearby, are not promoters. Thus, binding of the $E\sigma$ is not sufficient to define a promoter. To be a promoter, $E\sigma$ must bind and initiate transcription. **b** | A schematic representing the PRE promoter that does not have autonomous capability of binding $E\sigma$. This promoter shows little to no interaction with the $E\sigma$ in the absence of activator cII (green bubble). When cII protein is bound to its site, which overlaps the -35 element, it compensates the lack of consensus of this element, thereby allowing the $E\sigma$ interaction at PRE and allowing transcription of the *CI* gene (brown box)²¹⁰. Because there are promoters that are not bound autonomously by the $E\sigma$, this is not a necessary feature in the definition of promoter.

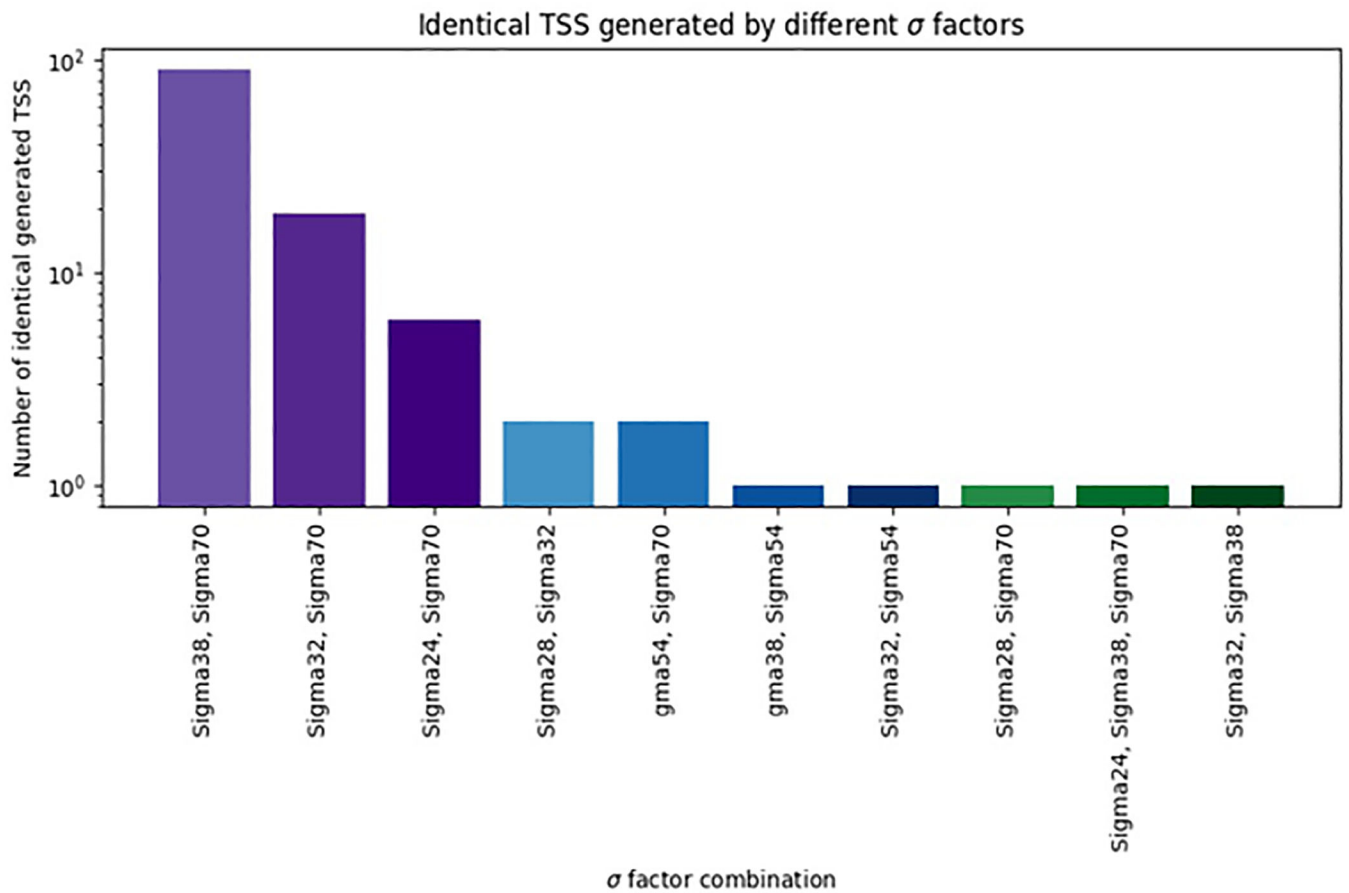


Figure 2 |
Relative numbers of TSSs generated by two or more σ factors according to the information in RegulonDB.

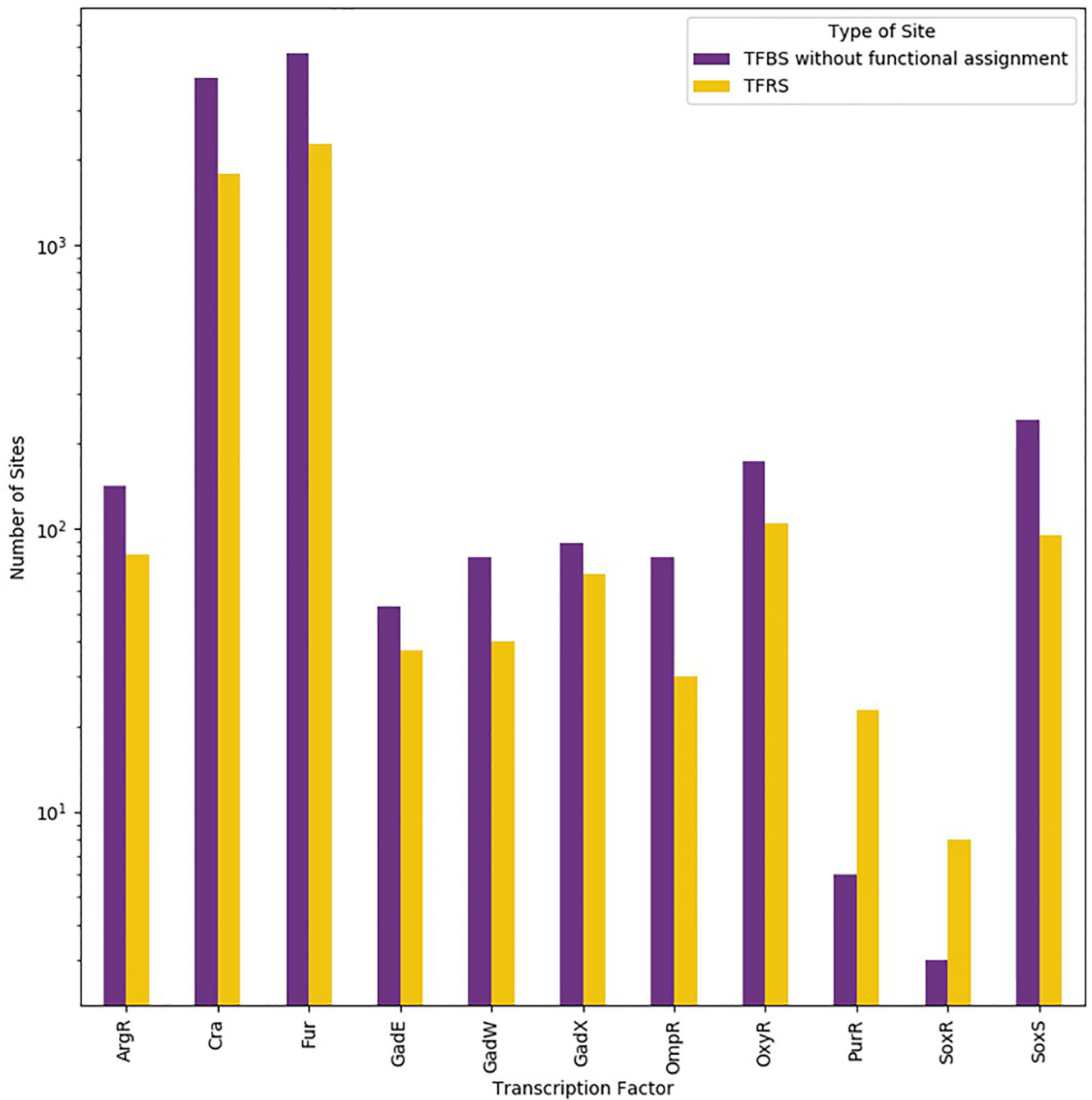


Figure 3 |. Number of transcription factor binding sites without functional assignment versus number of transcription factor regulatory sites in *E. coli*.

The sum of transcription factor binding sites (TFBSs) without functional assignment (purple) and transcription factor regulatory sites (TFRSs; yellow) represent the complete set of TFBSs. The figure was drawn using data from published chromatin immunoprecipitation (ChIP) experiments and data from classical experiments^{128,212–220}. Only TFs with non-functional sites found by highthroughput methodologies were included. A logarithmic scale is used to help visualize the disparate numbers of sites known for Fur and Cra and other TFs, such as DpiA and TrpR.

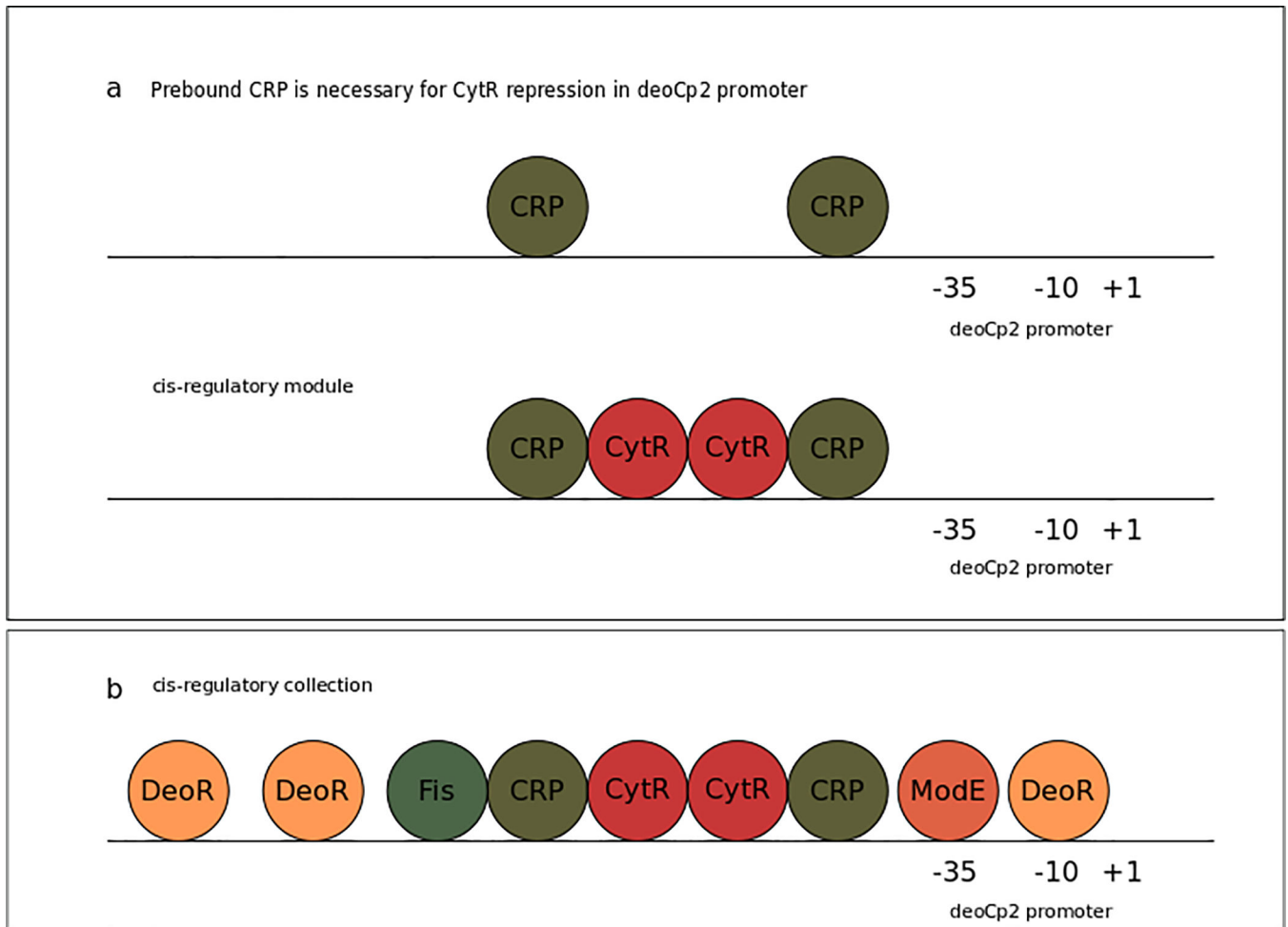


Figure 4 | *cis*-regulatory architecture of the promoter *deoCp2*.

The line represents the *deoCp2* promoter along with its transcription factor regulatory sites (TFRS). -35 and -10 elements and transcription start site (TSS; $+1$) are labelled, and the *cis*-regulatory architecture is represented by transcription factors (TFs; bubbles) bound to their corresponding TFRS. **a** | Cooperative regulatory interaction between CRP and CytR in the *deoCp2* promoter. CytR is recruited as a corepressor by pre-bound CRP²²¹. Since CytR necessarily requires pre-bound CRP to repress expression of the transcription unit downstream, these four sites form a TFRS module²²². **b** | The TFRS collection of promoter *deoCp2* is the complete set of TFRS known to regulate the *deoCp2* promoter. Although there might be indirect regulatory interactions among DeoR, ModE, Fis, CRP and CytR, the only direct and necessary interaction is the one between CytR and CRP^{223–225}. The other TFs act independently under different conditions on *deoCp2*, each with their own proximal sites.

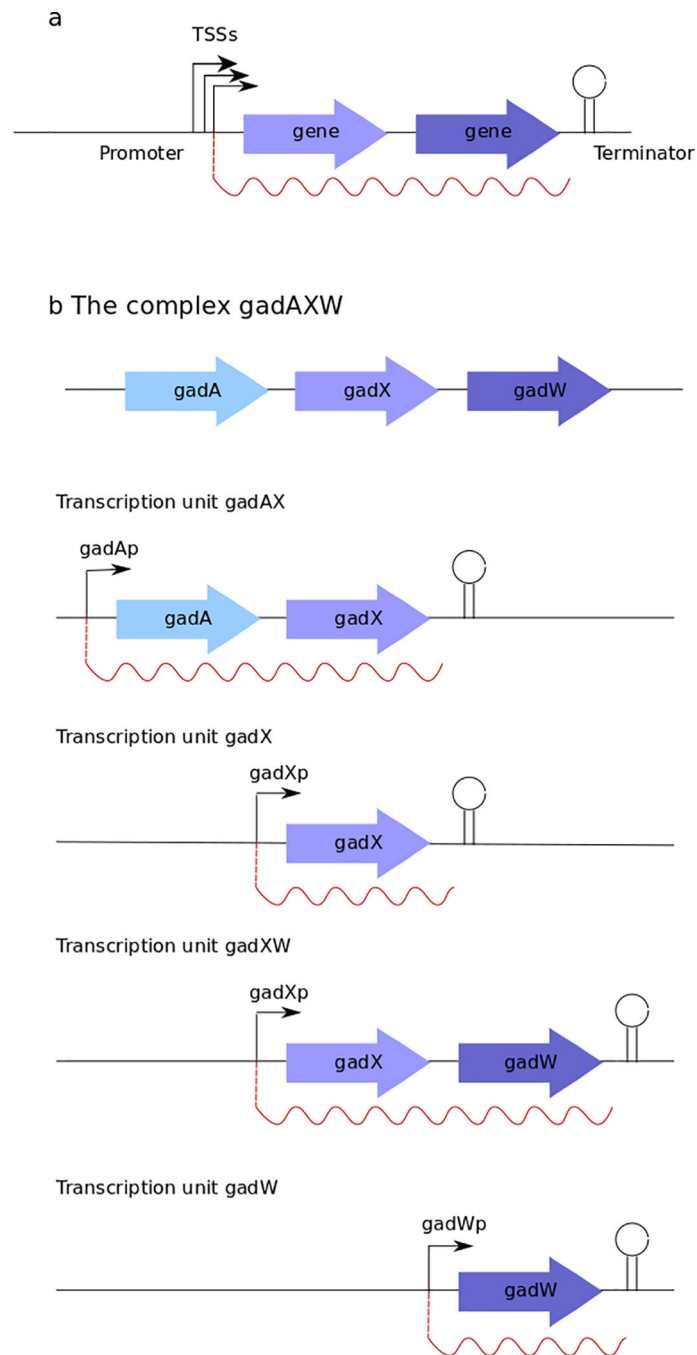


Figure 5 |. Transcription unit and operon schematic.

a | When different transcription start sites (TSSs) from the same promoter are not differentially regulated, they form a single transcription unit that is limited by, but not including, a single promoter and a single terminator. **b** | Schematic of the *gadAXW* complex operon. Several internal promoters and terminators enable different sets of genes to be co-transcribed in different combinations: *gadAX* by the *gadAp* promoter; *gadX* and *gadXW* by the *gadXp* promoter, and *gadW* by the *gadWp1* and *gadWp2* (not shown) promoters. Red wavy lines represent mRNAs. These promoters are subject to regulation by different sets of

TFs (not shown), so they are not all subject to the same signals. There are at least four operons in RegulonDB with no evidence of a single polycistronic transcript including all the genes of the operon.

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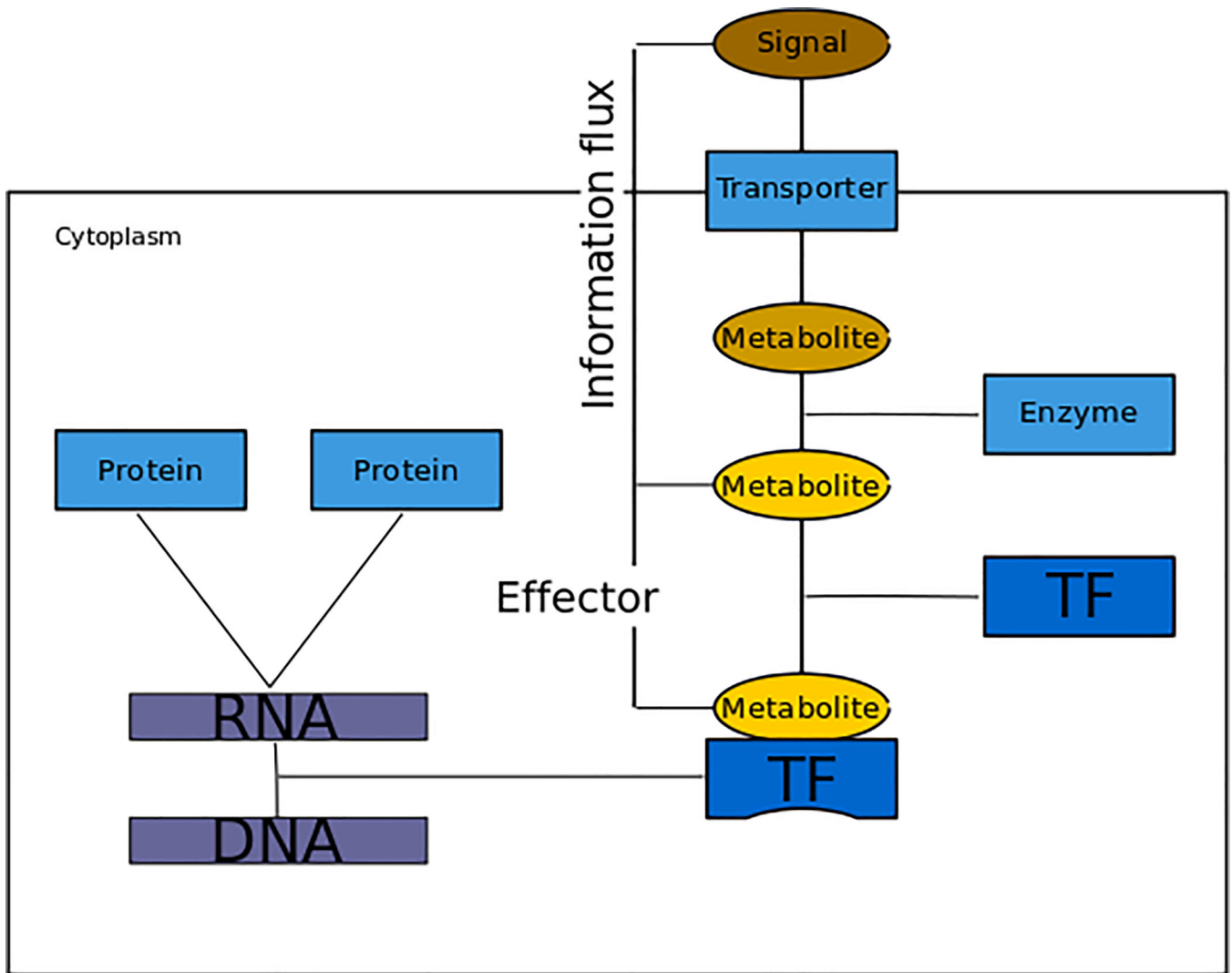


Figure 6 |. Schematic of signal and effector.

A cell reacting to the environment is represented. The rectangle represents the cell membrane, separating cytoplasm and the environment. A signal is represented as an environmental molecule (brown external bubble) that elicits a cellular response. The environmental molecule is introduced to the cell (brown internal oval) through a membrane transporter (blue square) and transformed by an enzyme into another molecule (yellow oval) that plays the role of effector by binding a transcription factor (TF) and modifying its ability to recognize its DNA binding sites. The concept of genetic sensory response units (SENSOR units) captures all the elements from the signal via the effector and regulation to the final regulated gene products as the response¹⁸⁶.