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Identifying global regulators in transcriptional regulatory networks in bacteria

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The machinery for cells to take decisions, when environmental conditions change, includes protein–DNA interactions defined by transcriptional factors and their targets around promoters. Properties of global regulators are revised attempting to reach diagnostic explicit criteria for their definition and eventual future computational identification. These include among others, the number of regulated genes, the number and type of co-regulators, the different σ -classes of promoters and the number of transcriptional factors they regulate, the size of the evolutionary family they belong to, and the variety of conditions where they exert their control. As a consequence, global versus local regulation can be identified, as shown for *Escherichia coli* and eventually in other genomes.

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Abbreviations

TF transcription factor

TU transcription unit

σ sigma factor

Introduction

The goal of modeling an entire *E. coli* cell has been set recently by a team of researchers [1]. We feel a more simplistic approach to modeling the behavioral repertoire of a cell would be to analyze a subset of interactions and molecular components of the cell responsible for cellular decisions at the level of transcription initiation. Transcription factors (TFs) have been described by François Jacob as ‘two-headed molecules’ [2] consisting of a DNA-binding site and an allosteric–metabolite interaction. Metabolites or covalent modifications link the sensing of extracellular stimuli with intracellular stimuli in the switch governing the expression or repression of genes, operons and regulons [3]. The relationship between sensing stimuli and deciding a pattern of expression involves the interplay of transporters, signal transduction mechan-

isms, thresholds of interactions, together with the organization of genes into operons, regulons and stimulons, and the control of chromosome structure [4,5].

Global regulators have been previously defined by Gottesman [6] on the basis of their pleiotropic phenotype and their ability to regulate operons that belong to different metabolic pathways. This definition excludes proteins involved in the essential cellular machinery. In this review, using what we currently know about transcriptional regulation in *E. coli*, we analyze different properties of TFs with a genomic perspective, and determine if a set of diagnostic criteria can be identified to provide an explicit way of distinguishing a global regulator from a local or dedicated regulator. The transcriptional network of *E. coli* is probably the best known for any cell, with 4405 ORFs identified [7] and an estimated 8%, or roughly 300 genes as predicted or known TFs [8]. RegulonDB, a database with information on transcriptional regulation and operon organization in *E. coli* [9], contains experimental evidence on regulation from 105 regulators affecting 749 genes. A large number of mapped promoters and sites, as well as operons, complete the description of around 20% of the regulatory interactions of transcription in the cell. The complexity of the network of currently known interactions is shown in Figure 1.

Number of genes regulated by transcription factors

It is surprising to know that seven regulatory proteins (CRP, FNR, IHF, FIS, ArcA, NarL and Lrp) are sufficient for directly modulating the expression of 51% of genes in *E. coli*, see Table 1, column 2. This large influence of so few regulators is consistent with the general statistical properties of this network, the connectivity of which follows a power-law distribution [10]. In a power-law network, very few nodes have a large number of connections, whereas many nodes interact with few others in a way that the ranked distribution of number of genes $N(k)$ as a function of their connectivity, in a log-log plot, decays linearly with a negative exponent [11]. At the other extreme, close to one-fifth of all TFs each regulates one or two genes.

Frequency of co-regulation

As mentioned above, the expression of many genes is modulated by only a few regulators; however, the regulation by multiple TFs occurs in 49% of genes and in most cases, it seems that a global regulator works together with more specific local regulators (Table 1, column 3). Specific examples of co-regulation include, the *melAB*

Figure 1

Overview of the transcriptional regulatory network in *E. coli*. Regulated genes are shown as yellow ovals, TFs are shown as green ovals and TFs considered to be global regulators are shown as blue ovals. The green lines indicate activation, red lines indicate repression and dark blue lines indicate dual regulation (activation and repression).

promoter by CRP and MerR [12], *ansB* promoters by CRP and FNR [13], *cytR* regulon by CytR and CRP [14], *proP2* promoter by CRP and FIS co-activation [15], *metY* by CRP and ArgR [16], among others [17]. Of the seven TFs mentioned above, only FIS regulates most of its genes (67%) in an independent manner. NarL and ArcA are at the other extreme as all their regulated genes are jointly co-regulated by other proteins, for instance in *moe* [18] and *hya* [19] operons. The strict co-regulation of modulons by NarL and ArcA with other TFs principally FNR and in a minor degree CRP and others, could be understood by the necessity of tight regulation between the sensing of redox potential balance between oxygen and alternative electron acceptors such as fumarate or nitrate (regulated by FNR), and the fine tuning regulation in aerobic or anaerobic responses by ArcA and NarL [20].

The mechanistic details of co-regulation vary. The active conformation of transcriptional factors before they bind to DNA in most, if not all cases, is a homo-multimeric oligomer [21]. Cooperative binding to DNA promoter operator regions increases the sensitivity for regulation [22,23]. For example, the same motifs of CRP involved in its interaction with the α subunit of RNAP are also used in its interaction with a specific TF, MerR [12]. It is not known if this is a unique accident in the recognition landscape, similar to the coincidence of the *gal* operator sequence and the sequence coding for the helix of the Gal regulator described years ago by Benno Müller-Hill [24]. If on the contrary, such promiscuity is a common property of specific interactions among regulators with many interactions, this would increase the possibilities for horizontal transfer of fractions of circuits and individual regulatory

Table 1

Summary of transcriptional interactions of major TFs, in the transcriptional regulatory network of *E. coli*.

Transcription factor	Genes regulated*	Co-regulators†	TFs regulated‡	Sigma factors§	Functional classes of genes regulated#	Family (members)¶
CRP	197	47	22	$\sigma^{70,38,32,24}$	48	CRP (2)
IHF	101	28	9	$\sigma^{70,54,38}$	26	HI-HNS (2)
FNR	111	20	5	$\sigma^{70,54,38}$	22	CRP (2)
FIS	76	15	4	$\sigma^{70,38,32}$	20	EBP (14)
ArcA	63	18	2	$\sigma^{70,38}$	17	OmpR (14)
Lrp	53	14	3	$\sigma^{70,38}$	15	AsnC (3)
Hns	26	14	5	$\sigma^{70,38,32}$	17	Histone-like (1)
NarL [¥]	65	10	1	$\sigma^{70,38}$	14	LuxR/UhpA (17)
OmpR	10	9	3	$\sigma^{70,38}$	5	OmpR (14)
Fur [¥]	26	8	2	$\sigma^{70,19}$	9	Fur (2)
PhoB	26	1	3	σ^{70}	9	OmpR (14)
CpxR	9	2	1	$\sigma^{70,38,24}$	5	OmpR (14)
SoxRS	9	10	3	$\sigma^{70,38}$	10	AraC/XylS (24)
Mlc [¥]	5	3	1	$\sigma^{9,32}$	3	NagC/XylR (7)
CspA [¥]	2	2	1	σ^{70}	2	Cold (9)
Rob ^{**}	7	8	2	$\sigma^{70,38}$	6	AraC/XylS (27)
PurR ^{**}	28	7	1	σ^{70}	10	GalR/LacI (13)

*Total number of genes regulated directly. †Number of different TFs with which at least a gene or TU is jointly co-regulated. ‡Number of regulated genes that codify for TFs. §List of σ factors of the regulated promoters. #Number of functional classes of the gene products regulated [44].

¶TF family and in parenthesis the number of members of the family. In addition to the seven global TFs considered here there are TFs suggested by

[¥]Babu and Teichmann, 2003, [42**] and ^{**}Shen-Orr *et al.*, 2002, [50**].

proteins, affecting in an important way the evolution of regulatory networks in bacteria [25].

Regulation of transcription factors and club co-regulation

In a process of decisions and information flux, the number of controlled or affected elements is not the only factor to be considered. A hierarchy of different levels of decision is natural to our understanding of how things get done [26]. One may consider that architecturally, the highest level in a hierarchy would be σ factors. Certainly, σ^{70} transcribes an enormous number of genes (830 genes compared with 31 for σ^{54}), as currently annotated in RegulonDB, and is therefore known for its housekeeping role. However σ^{54} , for instance, transcribes considerably less genes compared with those affected by a TF such as CRP (around 100 genes [27] compared with 200 genes). TFs amplify their range of control through the regulation of other TFs to encompass a set of indirectly regulated genes (Column 4 in Table 1). CRP is again by far, the transcription factor that regulates the most TFs (23 including itself). However, as TFs regulate other TFs, including themselves in feedback loops and complex relationships, we cannot assume a hierarchical organization of the network from these figures. Genes that are regulated by CRP also work with CRP in controlling other genes (Figure 2). In fact, 95% of regulators that are subject to control by CRP are also ‘colleagues’ involved in co-regulation. Of all the ‘colleagues’ with which CRP works, only 45% are subject to its direct control. These ratios might be different for each regulatory protein, but in general, as observed from Table 2, global regulators

work together with other global regulators (i.e. ‘club co-regulation’).

The dynamics of decision-making is a cooperative process of different subsets of the network put into action at certain moments. The grouping of co-regulated genes as a result of the different combinations of TFs working together in a given condition, provides the cell with the adequate flexibility to tune its transitions. This flexibility includes and could be partially analyzed in terms of the interactions and regulation among TFs.

Figure 2

Regulated genes and co-regulators of CRP. The intersection contains those TFs that are directly regulated by CRP and which work as co-regulators with CRP.

Table 2

Regulatory interactions between global TFs.

Global regulator	Global regulator work with (co-regulators)
CRP	IHF, FNR, ArcA, FIS, Hns, Lrp
IHF	CRP, FNR, ArcA, FIS, Hns, Lrp
FNR	CRP, IHF, ArcA, FIS, Hns, Lrp
Hns	CRP, IHF, FNR, ArcA, FIS
ArcA	CRP, IHF, FNR, FIS
FIS	CRP, FNR, ArcA, Hns
Lrp	CRP, IHF, FNR, Hns

Transcriptional requirements of σ factors

DNA-dependent RNA polymerase is the enzyme responsible for all cellular mRNA synthesis in *E. coli* [28]. In addition to the major housekeeping sigma factor σ^{70} , *E. coli* has six other σ factors [29,30]. Changes in the synthesis of σ factors, or competition of different σ factors to bind to the core RNA-polymerase, even if in a delayed fashion [31], cause different programs of gene expression to be induced and repressed. In some cases this is a global mechanism for differential gene expression during cellular development [32,33,34], as has been shown in the very well studied differentiation of *Bacillus subtilis* [35,36].

A single putative global regulator is capable of regulating genes transcribed by different σ factors (see column 5 of Table 1) and σ factors regulate genes subject to specific TFs. CRP, for instance, is able to regulate promoters transcribed by four different σ factors. Thus the cell has more than one alternative for partitioning its genes, either by hierarchical (if they are in fact hierarchical) relationships between the different regulatory genes, or in terms of classes of genes of different σ factor promoters. For example, a heat-shock or stationary-phase shift involves in addition to the global response by sigma factors, regulation and expression of genes involved in the precise carbon source, and nitrogen and oxygen availability. The complexity of different hierarchies and relationships shows us how the cell has encoded its actions into selected switches and groupings of genes. The cell is unlikely to have a single hierarchical perspective of regulatory networks, or a single structure-function association across species. It seems that in the same way as physiological properties of regulated genes do not depend on specific mechanisms or regulation, the decision-making distribution of tasks may be achieved differently in each organism.

TFs belonging to the σ^{54} class are specific and rarely overlap with TFs belonging to the σ^{70} class. Other than IHF and FNR, which have a repressor role, σ^{54} has its own separate class of activators (e.g. NtrC, NifA and XylR), which are clearly distinct. Certainly, the mechanisms and possibilities for regulation, in particular activation, are quite different to those available for

σ^{70} -like promoters [37]. The housekeeping polymerase σ^{70} transcribes most of the genes coding for TFs, suggesting that the decision-making machinery is able to induce changes in any direction when adapting to a change in environmental conditions. In fact, only six of the 75 known regulatory genes are transcribed exclusively by an alternative σ factor (*bolA* by σ^{38} , *csgD* by σ^{38} , *htgA* by σ^{32} , *hycA* by σ^{54} , *hypF* by σ^{54} and *zraR* by σ^{54}), none of which are considered as global regulators (see below).

Response to changes in environmental conditions

The second function of transcription factors is to sense changes in environmental conditions or other internal signals encoding changes. Bacteria constantly monitor extracellular physicochemical conditions, so that they can respond by modifying their gene expression patterns to adjust growth [38,39]. The link between changes in environmental conditions and changes in transcriptional regulation involves signal-transduction pathways, which may involve the direct production of an isomer such as allolactose [40], or a cascade of enzymatic modifications such as the activation of the nitrogen regulator in response to nitrogen limitation [27]. The precise link with regulatory proteins is achieved by small metabolites (i.e. products of active metabolism [41]). Half of the known and predicted transcriptional regulators in *E. coli* have predicted domains for the binding of small metabolites, whereas 10% have a CheY-like response regulator receiver domain that are phosphorylated by kinases in two-component signal transduction systems [42]. The role of both local and global regulators is to mediate precise activation or repression by sensing changes in specific metabolites. The only difference is that global TFs sense a larger number of growth conditions (shown in Figure 3). The concentration of cAMP must therefore be sufficient to enable CRP to perform its regulatory role [43]. As shown in column 6 of Table 1, the number of different functional classes [44] of the products whose genes are regulated by global regulators is clearly larger than the rest. Thus, not only do global regulators influence many other regulators and genes by transcriptional-specific interactions, they are also capable of sensing a larger number of environmental changes through specific metabolites.

Autoregulation and isolated transcription units

CRP is auto-regulated both positively and negatively. Lrp, FIS, IHF, Hns, FNR, Fur, PurR and SoxR among others, are negatively auto-regulated. In fact, auto-regulation is quite frequent in TFs. 55% (58 of 105) of all TFs known are auto-regulated, and of these 68% are negatively auto-regulated, 29% are positively auto-regulated (only PhoB of the TFs in Table 1) and 3% are auto-regulated both positively and negatively. Thus, auto-regulation is not limited to global regulators. Negative auto-regulation,

Figure 3

Global environment growth conditions in which TFs are regulating. To see the detailed list of conditions see RegulonDB page: http://www.cifn.unam.mx/Computational_Genomics/regulondb/SupMat/conditions. Numbers in brackets indicate how many additional TFs participate in the same number of conditions.

which has homeostatic properties [45], is predominant with TFs with high connectivity and is also dominant in the complete network [46].

CRP, FNR, ArcA, Lrp and Hns are isolated genes with a single promoter. NarL, OmpR, CpxR and PhoB are all part of two-component systems and are coded in transcription units (TUs) with their respective sensor proteins. The gene for FIS is part of a TU with two other genes and a putative dehydrogenase with unknown function. IHF consists of two subunits each of which are in different TUs, *himA* as a single gene and *himD* in an operon with two genes and three promoters including one that expresses *himD* only. It is interesting to note that global TFs tend to be transcribed uncoupled from the genes they regulate, which is not usually the case for specific TFs. Indeed, 70% of global TFs are encoded in operons or TUs with at least two genes, although independent promoters could still transcribe them. Precise theoretical analyses could help to understand the dynamic benefits of such coupled and uncoupled systems [47,48]. The fact that global TFs are uncoupled of the genes they regulate seems an inevitable consequence of regulating many genes. However, TFs that regulate a single or very few operons encoded by a gene adjacent to the regulated operon may well be the result of horizontal gene transfer, as it would not be advantageous to acquire a new operon conferring a new function if the operon is not expressed correctly [49].

Global transcription factors

Babu and Teichmann [42^{••}] and Shen-Orr *et al.* [50^{••}] have identified global regulators in *E. coli* (see Table 1). Seven of them satisfy our diagnostic criteria to be considered true global regulators; CRP, IHF, FNR, FIS, ArcA, Lrp and Hns. CRP is the master regulatory protein that senses the energetic status of the cell by cAMP levels [43,51[•]]. FNR and ArcA are directly related to energy

production by regulating respiratory modes. Lrp monitors the general nutritional state by sensing L-leucine concentration in the cell and adjusts metabolism to changing nutritional conditions [52]. FIS, IHF and Hns are DNA-binding proteins that regulate gene transcription by modulating DNA topology, which is dependent on the energy levels in the cell [51[•],52].

In a second level of hierarchy, we have identified NarL, Fur, Mlc and CspA, as also suggested by Babu and Teichmann [42^{••}], Rob and PurR, suggested by Shen-Orr *et al.*, [50^{••}], as well as PhoB, CpxR and SoxR. These DNA-binding proteins are less-connected nodes, but they are notably important for cell fitness. SoxR participates in oxidative stress, and regulates SoxS, which regulates a larger number of genes than SoxR. OxyR, PdhR, ModE, FlhA, CysB, DnaA, BolA, IciA and others proteins are also likely to be positioned at this level because they start regulatory cascades, although they do not satisfy other criteria as global regulators.

Furthermore, most global regulators described here belong to evolutionary protein families, which contain a low number of members. CRP, IHF, FNR, Lrp and Hns have been classified in families with three or less members [8]. FIS, ArcA, NarL, SoxS, Cpx, OmpR and PhoB belong to families with 14 to 27 members (Table 1, column 7). These families strongly depend on the similarity of the helix-turn-helix, thus being in a family with a small number of members would in principle permit a larger variation in sequence and number of the corresponding operator sites.

Additional levels of global coordination of gene expression in addition to TFs and σ factors include, for instance, methylation by Dam that overlaps with recognition sites for FNR and CRP [53], transient asymmetry expression of the regulatory network by chromosomal position [54],

as well as supercoiling in association with the overall energy level of the cell [31,34*,51*,55]. In fact, transcriptional interactions defines a subset of a larger network regulation including translation, RNA antisense [56], mRNA half-life [57,58**], transcription attenuation [59] and so forth.

Concluding remarks

This network of two-headed molecules that sense specificities of the environment, together with particular (overlapping) subsets of co-regulated genes that are expressed or repressed, describes what the cell has implemented to respond to the changes in the environment given its history in evolution. The armament for our understanding of regulatory networks, co-regulated groups, clusters and their overlapping genes, has recently increased beyond the operons, regulons and stimulons, to structurally and computationally defined motifs and modules of the network [50**,60**,61**], with dynamical properties of switches, oscillators, amplitude and frequency filters [62**], as well as with single-cell detection of transcriptional regulation and its stochasticity [63*,64**,65,66**]. Making biological sense in novel integrative ways seems to be closer at hand than ever before [67**,68]. The criteria proposed here and their evaluation can be computationally implemented, and thus contribute to other methods and strategies for reconstructing regulatory networks in different genomes [69*]. It is also important to evaluate whether the criteria proposed here for *E. coli* are of general validity for other organisms.

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