Article

Regulatory switches for hierarchical use of carbon sources in *E. coli*

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Abstract

In this work we study the preferential use of carbon sources in the bacterium *Escherichia coli*. To that end we engineered transcriptional fusions of the reporter gene *gfpmut2,* downstream of transcription-factor promoters, and analyzed their activity under several conditions. The chosen transcription factors are known to regulate catabolic operons associated to the consumption of alternative sugars. The obtained results indicate the following hierarchical order of sugar preference in this bacterium: glucose > arabinose > sorbitol > galactose. Further dynamical results allowed us to conjecture that this hierarchical behavior might be operated by at least the following three regulatory strategies: 1) the coordinated activation of the corresponding operons by the global regulator catabolic repressor protein (CRP), 2) their asymmetrical responses to specific and unspecific sugars and, 3) the architecture of the associated gene regulatory networks.

Keywords *Escherichia coli*; transcription factors; carbon sources; hierarchical use.

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1 Introduction

The way bacteria use different carbon sources (Monod, 1942) has been studied for a long time, in which *Escherichia coli* has been the favorite model organism. We learned from the very beginning that glucose is the carbon source supporting the fastest growth on this bacterium (Walker et al., 1934). This sugar is also the preferred one if bacteria are exposed to a mixture of carbon sources. It seems that *E. coli* uses carbon sources on the basis of "best food served first". The molecular mechanisms behind this operating principle are various, the best-known ones are: inducer exclusion, local or dedicated transcriptional regulation, global transcriptional regulation, small RNAs, and catabolite repression.

Inducer exclusion (Jones-Mortimer and Kornberg, 1974; Chen et al., 2013) takes place when, in the presence of glucose or other PTS sugars, the unphosphorylated $EIIA^{Glc}$ (part of the PTS system) binds to and

stabilizes the resting state of non PTS-sugar transporters, inhibiting the transport (and use) of alternative carbon sources.

Local or dedicated transcriptional regulation operates at the initiation of gene transcription of sugars catabolic operons. These operons are normally subject to repression by at least one specific regulator, whose derepression occurs when the corresponding specific sugar is available and binds to it. This binding causes the effector-repressor complex to unbind from the operator zone, which is a necessary condition for the corresponding operon to become active (Jacob and Monod, 1961; Sellitti et al., 1987).

Global transcriptional regulation. The complementary condition for the transcription of sugar catabolic genes is given by the activity of the global regulator CRP (catabolic repressor protein or cAMP regulatory protein). CRP becomes active when bound by cyclic adenosine monophosphate (cAMP). The cAMP-CRP complex is then capable of recruiting RNA polymerase to promoter zones of catabolic operons so their transcription is started if no repressor is present. Hence, a condition for the transcription of catabolic operons is that high cAMP levels are present. High cAMP levels are in general achieved in the absence of glucose although, as mentioned below, it could be the result or a wider physiological status (Gottesman, 1984; Martínez-Antonio and Collado-Vides, 2003).

Small RNAs (sRNA). Arguably, the best known sRNA is the multi-target Spot42, which inhibits the translation of at least 14 genes, mostly related to the use of non-PTS sugars. Spot42 is activated by cAMP-CRP and together form a coherent feed-forward loop to avoid use of non-PTS sugars when the preferred sugars are available (Beisel and Storz, 2001; Wright et al., 2013).

Catabolite repression (Magasanik, 1961; Görke and Stülke, 2008), a physiological concept so-named by Boris Magasanik as a generalization of the "glucose effect" described many years earlier (Cohn, 1957). It was derived after observing the repression, when glucose is present, of catabolic enzymes specific for carbon and nitrogen metabolism. This phenomenon was related to cAMP levels that increase when poor carbon sources are present in the milieu (Epstein et al., 1975). cAMP is synthesized by the CyaA enzyme, which is activated by phosphorylated $EIIA^{glu}$ but requires an additional unidentified factor (Park et al., 2006). It was postulated that a derived catabolite of carbon sources (the repressor catabolite) is the responsible to trigger cAMP syntesis. Only recently, a high-throughput proteome analysis (studying carbon, nitrogen and sulfur sources metabolism) in *E. coli* revealed that cAMP levels are diminished by α-ketoacids (mainly by oxaloacetate) through the inhibition of adenylate cyclase, the enzyme responsible for cAMP synthesis (You et al., 2013). This explains how this central metabolite is balancing the overall bacterial physiology throughout the nitrogen/carbon metabolism (Rabinowitz and Silhavy, 2013).

Here we present a study that copes with the activities of the promoters of specific catabolic regulators, which in addition to self-regulation, respond to the global regulator CRP (points 2 and 3 above). These locals and the global regulator operate together to regulate transcriptional initiation in *E. coli* catabolic operons for the transport and use of carbon sources other than glucose.

We tackle the question of how bacteria decide to consume alternative carbon sources, focusing in L-arabinose, D-sorbitol and D-galactose. The regulation, transport and first catabolic steps in the metabolism of these sugars are depicted in Fig. 1. As we can see, not only the corresponding genes are activated by CRP, but they are also repressed by specific transcription factors. We investigate in this work the promoter activities of these specific regulators. Importantly, all of them use the transported sugars as signal effectors to modulate their activities. The signal sugar binds to the repressor and unbinds it from the operator zone, thus allowing transcription of the corresponding genes. Finally, all the promoters here analyzed require of the housekeeping σ^{70} to be transcribed so this is not a variable to consider in this study.

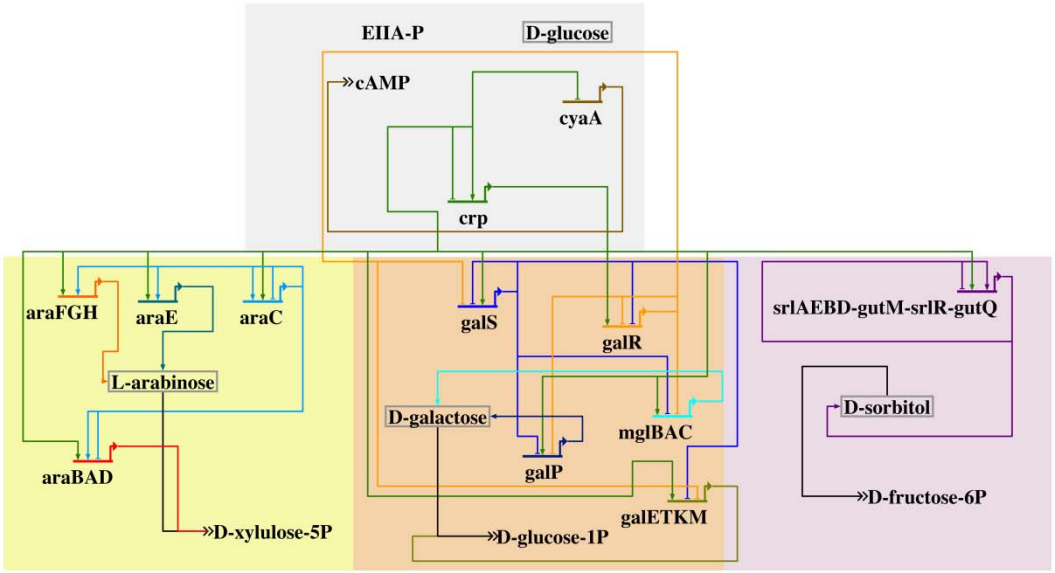

Fig. 1 Regulatory network controlling the use of the carbon sources employed in this study. It is show the module corresponding to the global regulator CRP. cAMP, a co-activator of CRP, is synthesized via CyaA when glucose is absent. The arabinose module includes the dual regulator AraC, which transcriptionally regulates the arabinose transporter genes AraE (low affinity) and AraFGH (high affinity). AraC also regulates the genes of enzymes isomerase (AraA), ribolukinase (AraB) and epimerase (AraD), which metabolize arabinose to D-xylulose 5-P. The galactose module has two repressors, GalR and GalS, in different transcription units. In absence of galactose they repress the genes for galactose transporters GalP (low affinity) and MglBAC (high affinity), as well as those for the enzymes GalK (galactokinase), GalT (uridiltransferase), and galM (epimerase), which metabolize galactose to glucose 1-P. The sorbitol module is also regulated by two transcription factors, SrlR and GutM, encoded in the same operon, which also includes genes for high affinity transporter (SrlAEB) and for the enzymes SrlD (dehydrogenase) and GutQ (isomerase), which transform sorbitol to fructose 6-P. This figure was created using the BioTrapestry software (Longabaugh et al, 2009).

2 Material and Methods

2.1 Strains

In all our experiments we employ *Escherichia coli* K-12 MG1655 strain and derivatives harboring the different transcriptional fusions show in Table 1. Most of the used transcriptional fusions were taken from a collection reported previously (Zaslaver et al., 2006). However, we rebuilt the transcriptional fusions for *gutM* and *crp* promoters in order to include regulatory sites for transcription factors not comprised in fusions from the collection. We realized the necessity of such regulatory sites by inspecting the transcription-factor binding sites reported in RegulonDB (Salgado et al., 2013). These last fusions were engineered by amplifying (through PCR and specific primers) the corresponding regulatory regions, cloning the resulting DNA fragments on pUA66 with the aid of the BamH1 and XhoI restriction sites, and verifying the construction by means of DNA sequencing.

2.2 Bacterial growth

For strain maintenance we routinely used LB medium and for experimental tests we used M9 medium, supplemented with sugars as indicated. Also when indicated, we added kanamycin (Km) 50 μg ml-1. Pre-inoculates were grown overnight in 5 ml of LB medium at 37 ºC with agitation (200 rpm). Next, the

cultures were diluted 1 : 100 in 150 µl of fresh M9 media in micro-titer plates of 96 wells and incubated for 12 h with agitation (250 rpm) at 37 ºC. We supplemented M9 with 0.4% or 0.03% of glucose, and 0.2% of one or two alternative sugars as specified. We followed bacterial growth, by measuring OD595nm, and fluorescence (535 nm) every hour in a Perkin Elmer Victor X3 plate multi-lector.

Table 1 Regulatory regions employed on the transcriptional fusions.

Promoter fusions	*E. coli* chromosome coordinates	Designed primers* 5´-3´	Region size	Cloning vector	Reference
araCp::gfpmut2	69973-70452		479bp	pUA66	Zaslaver et al., 2006
crpp::gfpmut2	3483776-3484200	F:tgatgactcgaggcggatt c R:tggcaatgagacaggatc ca	424bp	pUA66	This study
galSp::gfpmut2	2239619-2239844		225bp	pUA139	Zaslaver et al., 2006
galRp::gfpmut2	2973960-2974698		738bp	pUA66	Zaslaver et al., 2006
gutMp::gfpmut2	2823533-2823932	F:cttgctgctcgaggcggca a R:ccatccggatccacacctc tccgc	399bp	pUA66	This study
srlRp::gfpmut2	2826905-2827074		169bp	pUA66	Zaslaver et al., 2006

*Underlined nucleotides define restriction sites for XhoI and BamH1 endonucleases on forward and reverse primers.

2.3 Data acquisition and processing

The raw numerical data obtained from the Victor X3 plate multi-lector consisted of discrete measurements of optical density (OD) and fluorescence (GFP) versus time along the growth curves, with a sampling frequency of 1 hr^{-1}. Although enough to provide an overview of the time evolution of variables OD and GFP, such sampling frequency is too low to perform more refined quantitative analyses. For that, it is necessary to find a function that fits the experimental data. Since the generalized logistic function is a widely used sigmoid function for growth modeling we decided to employ it. In all cases we found that it fits both the growth curves and the GFP profiles with correlation factors higher than 0.99. The functions used to fit the OD and GFP profiles are:

$$OD(t) = a_1 + \frac{k_1 - a_1}{\left(1 + q_1 e^{-b_2 t}\right)^{1/v_1}}, \quad (1)$$

$$GFP(t) = a_2 + \frac{k_2 - a_2}{\left(1 + q_2 e^{-b_2 t}\right)^{1/v_2}}, \quad (2)$$

in which a_i, b_i, k_i, q_i, and v_i (i = 1, 2) are fitting parameters. Zaslaver et al. (2006) and Martínez-Antonio et al.

(2012) have argued that promoter activity is proportional to (d*GFP*(*t*) / d*t*) / *OD*(*t*). Thus, after finding the best fitting parameters we differentiated function (2) and divided the result by eq. (1) to compute the promoter activity in each case.

For every experimental condition and for every transcriptional function we periodically measured the values of optical density and green fluorescence in triplicate, computed the corresponding average values, and respectively fitted to Eqs. (1) and (2), and computed the promoter activity level as explained above.

In our experiments we could observe that the *crp*, *galR* and *srlR* promoters were unresponsive under all the tested conditions (data not shown). The invariable of *crp* promoter activity might be explained because it is the most global regulator in *E. coli*. Not only *crp* regulates itself, but it is also subject to dual regulation by another global regulator: FIS (factor for inversion stimulation). In the case of *galR* and *srlR,* the reason why they present constant low expression levels may be that they are constitutively expressed; up to date no regulator is known for these genes. Due this unresponsiveness and for the sake of clarity we excluded the results corresponding to these promoters in the fore coming sections.

3 Results

3.1 Different carbon sources support the grown of *E. coli* differentially

Our first objective was to analyze how the different carbon sources under study support the growth of *E. coli*. For this, we followed the progression of *E. coli* cultures growing in M9 minimal medium added with L-arabinose, D-sorbitol and D-galactose, both separately and in dual combinations. The growth profiles show in Fig. 2A confirm that glucose is by far the sugar that best supports *E. coli* growth. The hierarchical order of sugars in terms of their capacity to sustain cell growth is as follows: glucose > arabinose > sorbitol > galactose. When combinations of two alternative sugars were used, the bacterial growth rate almost equated that of glucose during the exponential growth phase. On the other hand, with all the sugar combinations, the maximal bacterial population density surpassed that of glucose alone. The decreasing order of alternative sugar combinations in terms of the exponential growth rate they are capable of sustaining is: arabinose+sorbitol > arabinose+galactose > galactose+sorbitol. In these experiments, glucose was set at a limiting amount (0.03%) from the very beginning to clearly distinguish the time at which *E. coli* starts using alternative carbon sources (Fig. 2A). We observed a differential growth of cultures with not limitation as compared with those limited on glucose as early as 3.5 hours after the start of the experiment. However, a careful observation on the alternative-sugar catabolic-operon promoter activity reveals that they become active after 2 hours of the experiment beginning (see below).

3.2 Glucose limitation triggers foraging alternatives

Our second objective was to study the dynamics of the alternative-sugar catabolic-operon promoters under glucose exhaustion conditions. Specifically, we were interested in the following scenarios: 1) when glucose is limiting from the culture at the very beginning and, 2) when glucose is exhausted after a normal period of bacterial growth. For that purpose we engineered specific reporters for relevant transcription factors (Table 1). These reporters were built by transcriptionally fusing each promoter to gene *gfpmut2*, and promoter activity was estimated by measuring fluorescence along the bacterial growth curves (Zaslaver et al., 2006). We made sure that the presence of the vector and genetic constructions were not detrimental for *E. coli* growth before the assays.

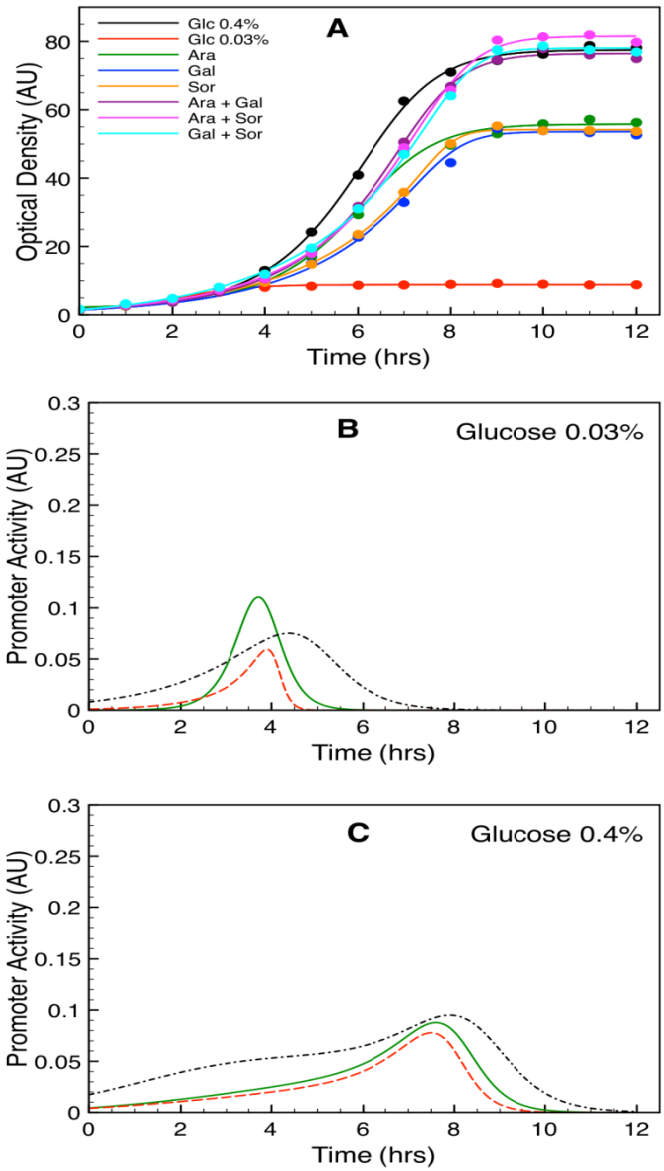

Fig. 2 Carbon source consumption by *Escherichia coli*. **A)** Wild-type *E. coli* growth curves (experimental data and best fitting generalized logistic functions) while cultured with different carbon-source concentrations and combinations. **B)** Transcription-factor promoter activities under glucose limitation conditions (M9 + 0.03% glucose). **C)** Transcription-factor promoter activities during a normal course of *E. coli* growth (M9 + 0.4% glucose). The color code in all graphs is as follows: *araC*, black; *gutM*, green; *galS*, red.

The results of these experiments can be summarized as follows. The time when alternative promoters are maximally active depends on the time at which glucose is exhausted. When glucose is limiting from the beginning of the experiment, the alternative-sugar catabolic-operon promoters start activating as soon as 2 hours after the experiment start, they reach their maximal of activity around the hour 4, and their activity starts declining thereafter (Fig. 2B). Contrarily, if there is a considerable amount of glucose at the culture beginning, the alternative-sugar catabolic-operon promoters become active only after glucose has been presumably exhausted, with the exemption of *araC* promoter that shows some activity during all the experiment. The three examined promoters reach their maximal activity about 8 hours after the beginning of the experiment (Fig. 2C).

The promoter behaviors reported in Figs. 2B and 2C, in absence of specific sugars in the milieu, can be explained by the activity of the master regulator CRP which only becomes active and turns up its target genes (among others the ones corresponding to the here studied transcription factors) when glucose is exhausted, and supposedly, when cAMP production is increased. Interestingly, no matter how fast glucose is exhausted, the studied promoters start showing some sign of activity about 2 hours after the cultures' start. Finally, under conditions of high initial glucose levels, not only all promoters reach their maximal activity at roughly the same time, but also their maximal activity levels are quite similar. When the initial glucose concentration is low, the maximal levels are dissimilar, although they are reached at similar times. However, it is important to emphasize that promoter activity is inversely proportional to bacterial density and that bacterial density (estimated by means of optical density measurements) is very low when glucose levels are initially low. All this implies that the obtained maximal promoter activity levels are not as reliable as those corresponding to high initial glucose concentration. Having this in mind it is remarkable that the maximal promoter activity levels have the same order of magnitude in all cases, see Figs. 2B and 2C.

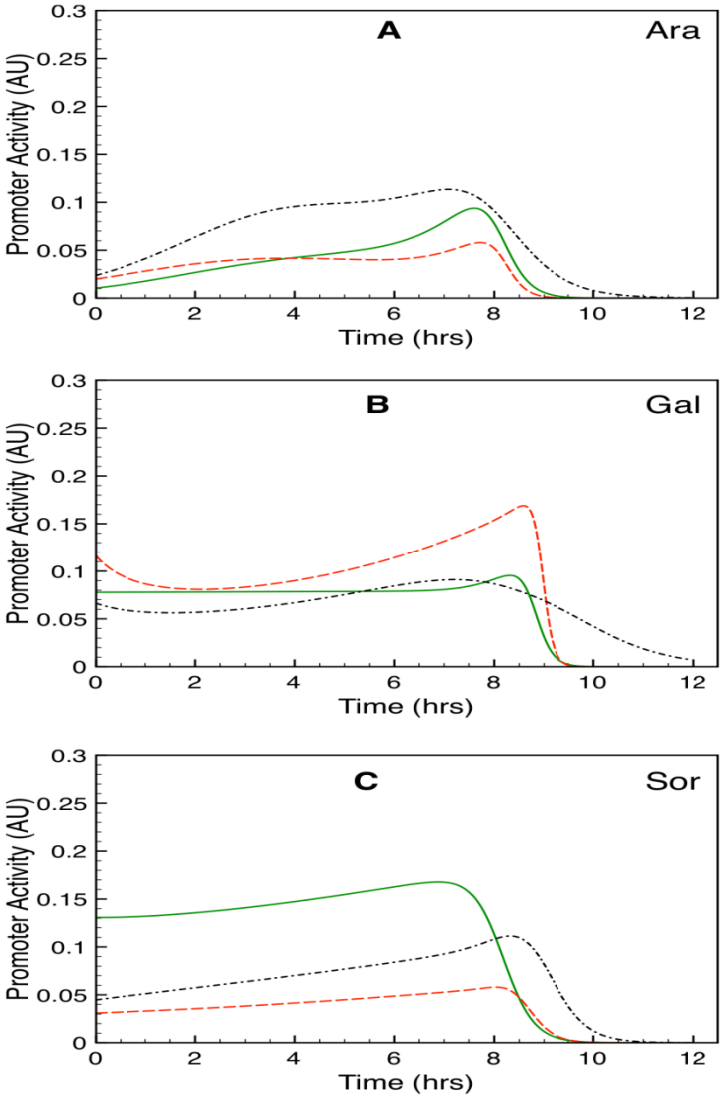

Fig. 3 Transcription-factor promoter activities as response to the presence of alternative carbon sources. **A)** Promoter activities in the presence of L-arabinose. **B)** Promoter activities in the presence of D-galactose. **C)** Promoter activities in the presence of D-sorbitol. The alternative sugars were supplemented at 0.2% (M9 + 0.03% glucose + 0.2% each alternative sugar). The color code in all panels is as follows: *araC*, black; *gutM*, green; *galS*, red.

3.3 Promoter specificity for sugar effectors

We further investigated the specificity of promoter response to different sugars. To that end we grew *E. coli* in M9 medium with a limiting amount of glucose plus different carbon sources, and measured the activity of the previously mentioned transcription-factor promoters. The results are reported in Fig. 3 in which the promoter activities are plotted vs. time under different conditions.

We found that each transcription-factor promoter primarily responds to the sugar whose catabolic operon it controls. However the individual effects vary in amplitude as follows. The largest effect is that of sorbitol on *gutM*, whose maximal activity is about 2.4 times larger the one caused by glucose exhaustion solely; then we have the effect of galactose on *galS* that shows a 1.8 fold increased activity; and the smallest response is that of *araC* to arabinose, with an increase of 1.3 in the promoter activity.

We could also observe a cross-regulation effect between signals (sugars) and the expression of transcription factors. This may allow transcription factors to display asymmetrical responses to specific and unspecific sugars. We can see that arabinose has a slight positive effect on *gutM* promoter, while it inhibits the expression level of *galS* promoter by about 40%. Regarding sorbitol, it increases by about 40% the expression level of promoter *araC*, and inhibits by about 30% the expression of promoter *galS*. Finally, galactose has no noticeable effect on *araC* promoter, but increases by about 20% the expression level of *gutM* promoter. The above-discussed results are summarized in Fig. 4.

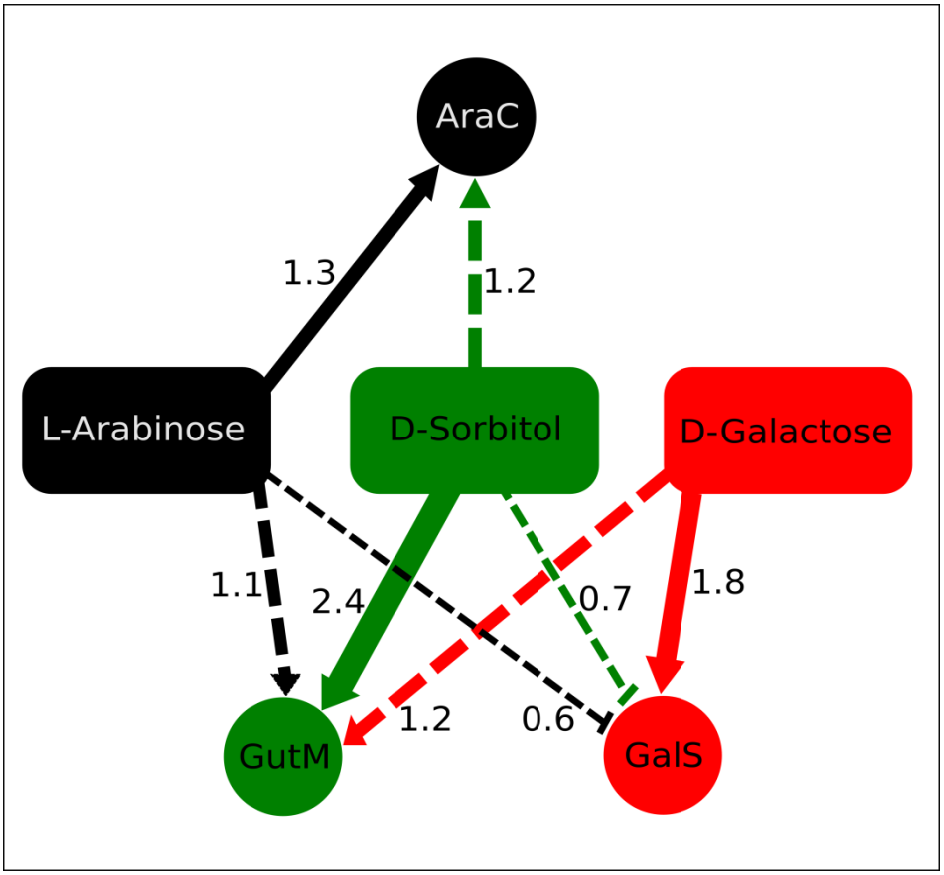

Fig. 4 Asymmetrical response of transcription factor promoters to sugar signals. The values next to the arrows indicate the percent of response of each promoter to every one of the three alternative sugars, as compared to the effect caused by glucose exhaustion solely. Continuous lines indicate the response of transcription factor to their effector sugar, while dashed lines indicate cross-response to the presence of other sugars.

Note from Fig. 4 that the promoter that is most responsive to sugars other than its own one is *araC*, followed by *gutM* and *galS* promoters. This behavior is interesting because a partial turn on of promoters due to non specific stimulus is an indicator of a putative conditioned behavior already observed in sugars consumption in *E. coli* (Tagkopoulos et al., 2008). Our results suggest that the promoter most prone to conditional behavior is that of *araC*.

Finally, the sugar that most enhances the activity of transcription-factor promoters other than the one specific to it is galactose. This suggests that, in agreement with (Liu et al., 2005), the worse a carbon source that sustains bacterial growth, the more it positively affects the activity of alternative-sugar catabolic operons.

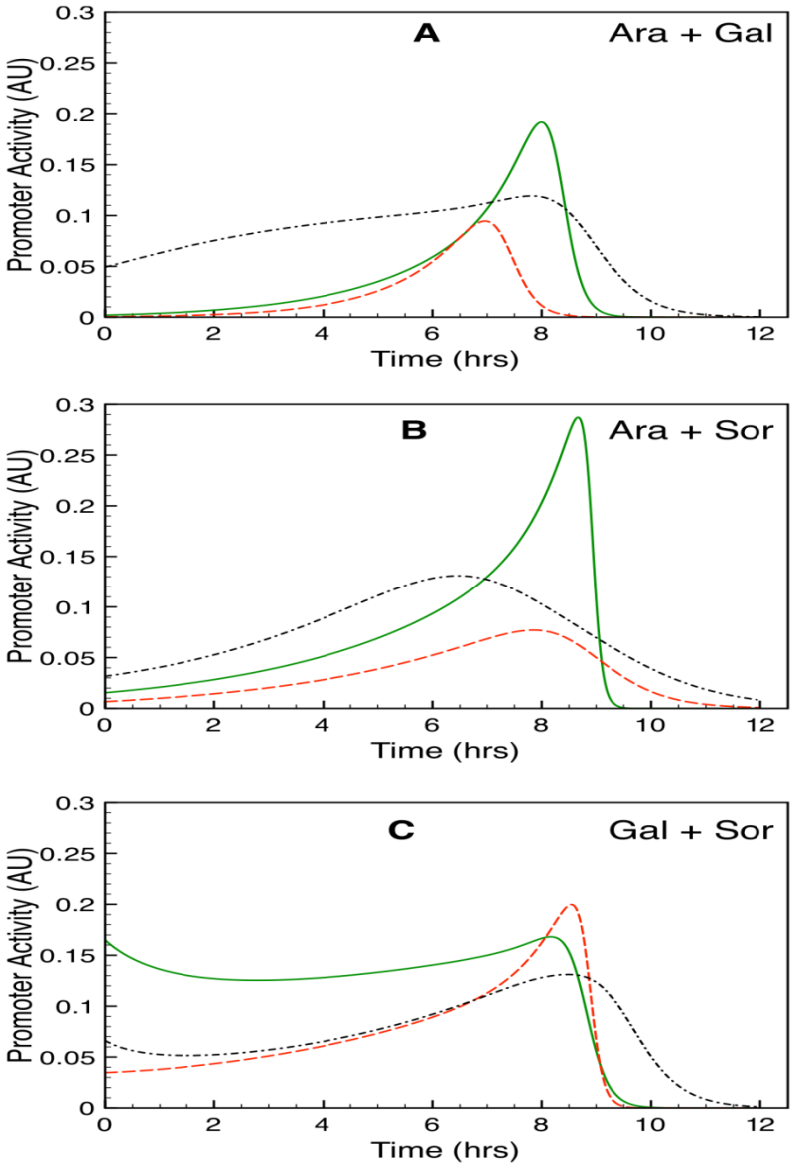

Fig. 5 Transcription-factor promoter activities in the presence of pairs of alternative sugars (M9 + 0.03% glucose + 0.2% sugar 1 + 0.2% sugar 2). **A)** Promoters activities in the presence of arabinose and galactose. **B)** Promoter activities in the presence of arabinose and sorbitol. **C)** Promoter activities in the presence of galactose and sobitol. The color code in all panels is as follows: *araC*, black; *gutM*, green; *galS*, red.

3.4 Promoter activities reflect carbon-use hierarchy

We finally performed experiments in which bacteria were grown in the presence of a limiting quantity of glucose (0.03%) and a mix of two alternative sugars (0.2% each). The rational behind this experiment was that once glucose is exhausted, *E. coli* should be forced to consume one of the two alternative sugars present in the milieu, and that this decision might be evidenced by the activity of the promoter associated to the transcription factor regulating the catabolic operon of the sugar of choice. The results of these experiments are reported in Fig. 5.

Notice that whenever bacteria are cultured in the presence of arabinose, promoter *araC* becomes active before the other two. The explanation of this observation is straightforward under the assumption that arabinose is preferred by bacteria over sorbitol and galactose.

Note from the arabinose + galactose experiment (Fig. 5A) that the positive influence of galactose upon *galS* is capable of completely counteracting the negative influence of arabinose. This is consistent with the supposition that arabinose is consumed before galactose by bacteria. It is also interesting that the rather small positive effects that both arabinose and galactose individually have on *gutM* boost each other to render a combined over-expression of more than 100%. A detailed observation of the curves in Fig. 5B (arabinose + sorbitol experiment), reveals that their amplitudes completely agree with the interaction scheme in Fig. 4.

In Fig. 5C (sorbitol + galactose experiment) we can see that the negative effect of sorbitol on *galS* is fully counteracted by the positive effect of galactose. However, the maximum activity of promoter *galS* is posterior to that of promoter *gutM*. This suggests that sorbitol is consumed before than galactose by bacteria. Furthermore, the maximal expression levels correspond to what one would expect from the interaction scheme in Fig. 4.

In summary, our results suggest that the investigated alternative sugars are consumed in the following order: arabinose, sorbitol and galactose. Moreover, the maximum expression levels in the experiments with two alternative sugars agree with the interaction scheme reported in Fig. 4, except for the expression of promoter *gutM* in the arabinose + galactose experiment. It seems that arabinose and galactose synergically make promoter *gutM* increase its expression level by more than 100%.

3.5 CRP as a global coordinator for carbon metabolism

All the transcription-factor operator regions here analyzed include a DNA-binding site for CRP, in addition to being self-regulated. In the previous sections we studied the contribution of specific catabolite signals to the activity of their local regulators. Thus, to have a complete picture it is necessary to test the effect of CRP (and indirectly that of cAMP) on the promoter activities of these local regulators. To this end we used a CRP mutant strain (Baba et al., 2006) as a receptor of the transcriptional fusions analyzed before, and measured growth and promoter activities when glucose is limiting at the beginning (0.03%) and at the end of the culture (0.4%), see Fig. 6. A first observation is that deletion of *crp*, although not essential, has negative effects on the bacterial growth rate (Figs. 6B vs 6A). Note that the culture final OD decreases as compared with that of the strain with an intact *crp* gene (comparable on 0.4% glucose). This could be explained by taking into consideration that CRP is a global coordinator of *E. coli* physiology, which regulates more, that 30% of all the genes with known regulation in this bacterium. However, the negative effect on growth is more pronounced in the strains harboring the transcriptional fusions of *gfp* with *galS* and *gutM* promoters that that with *araC*. We do not have a consistent explanation for this observation.

Regarding the promoter activities in the absence of *crp,* when glucose is depleted at the beginning of the culture (0.03%), the promoters activities changed as follows, as compared with the intact-*crp* strain: the *araCp* activity profile changed neither its amplitude nor the time at which the maximum value was achieved, yet the profile is now narrower; the maximal *gutMp* activity level was doubled, although it was retarded by more than

one hour; the *galSp* activity profile became wider and its maximal level decreased by about 50% (Figs. 6D vs 6C).

On the other hand, when glucose is exhausted at the end of the culture (0.4%) all the promoter activities are diminished and retarded except that of *araCp* (Figs. 6F vs 6E). Together, these observations indicate that CRP contributes to the fitness and performance of bacterial growth and coordinates the response of alternative regulatory machinery for the use of alternative carbon sources in *E. coli*, although it seems not to be essential for the transcriptional response of local repressors.

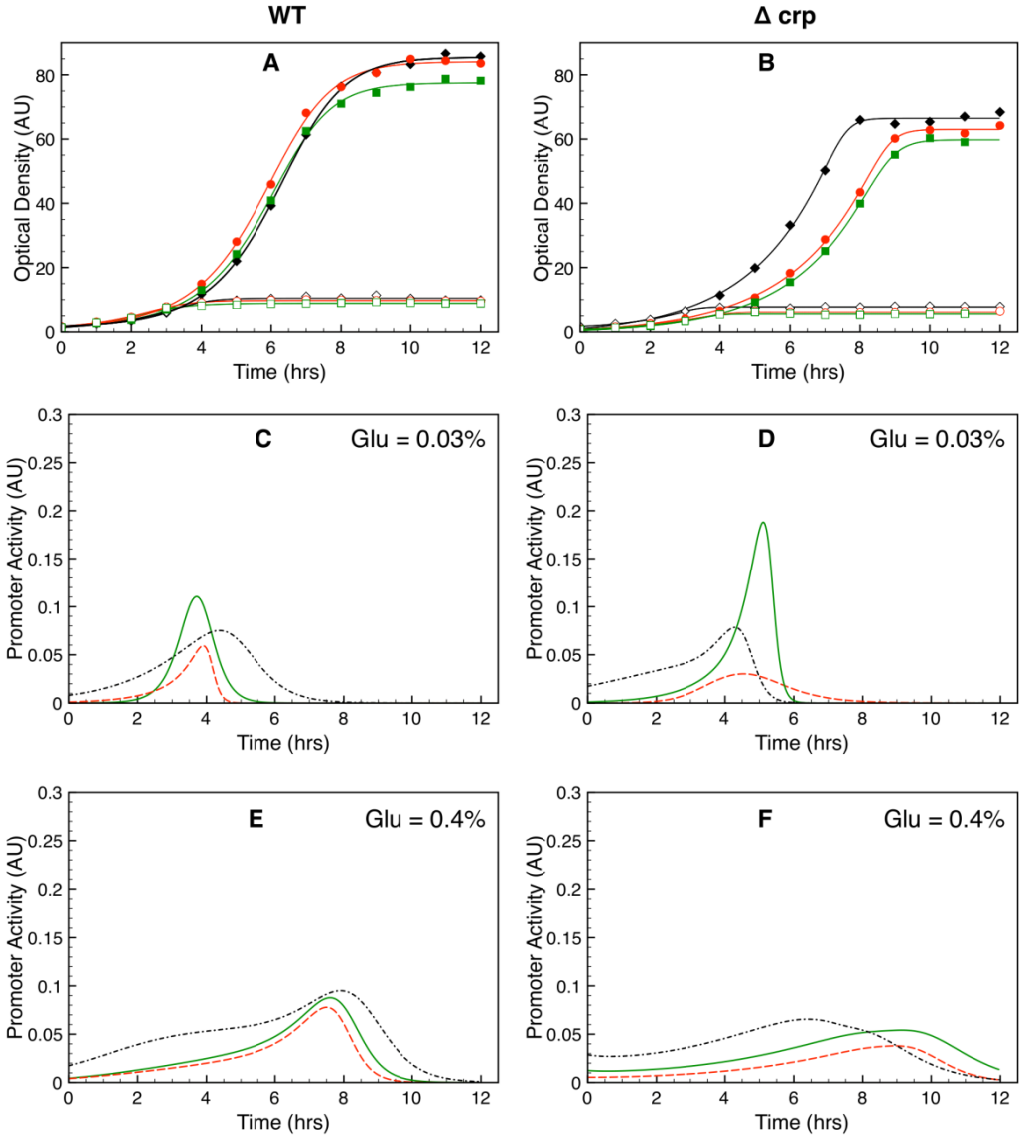

Fig. 6 Effect of CRP on the promoter activity of various catabolic repressors. Growth of bacteria harboring the transcriptional fusions on wild type background (A) and on *crp* deletion (B). Solid (empty) symbols correspond to a glucose concentration of 0.4% (0.03%). Promoter activities with glucose limited at the beginning of the culture on WT (C) and on Δ*crp* mutant (D). Promoter activities without glucose limitation at the beginning of the culture on WT (E) and on *crp* deletion (F). The color code in all panels is as follows: *araC*, black; *gutM*, green; *galS*, red. (C) and (E) are the same as Figs. 3B and 3C but are repeated here for the sake of clarity.

3.6 Could the architecture of regulatory circuits be responsible of this hierarchical behavior?

Given the displayed activities of specific regulators for alternative sugar consumption we decided to analyze

the operator regions of the corresponding promoters, Fig. 7. It has been proposed that CRP recruits the *E. coli* RNA polymerase differentially to distinct promoters (Parkinson et al., 1996; Lee et al., 2012). As a result, CRP has different modes of activation. Briefly, in Class I activation, CRP is bound to an upstream site of the -35 element of promoters and contacts RNA polymerase throughout their αCTD subunit. In Class II activation, CRP binds to a site that overlaps the -35 element and contacts RNA polymerase throughout the domain 4 of σ^{70}subunit. Of the promoters here studied *araCp* correspond to the class I whereas *galS* and *gutMp* correspond to the class II (Fig. 7). In addition, there are some other differences on the promoters' architecture. For instance, *araCp* regulation involves a DNA loop by interaction of AraC dimmers (Gallegos, 1997) and the binding sites for CRP and AraC fall inside this loop. In the case of *gutMp*, although there is evidence of regulation by GutM and SrlR, the DNA binding sites for these regulators have not been identified. However, it seems that SrlR repress the transcription of *gutMp* and GutM positively auto-regulates it (Yamada and Saier, 1988). Regarding *galSp*, it has a DNA binding site that seems to be the target of two repressors: GalS and GalR. These regulators bind such a site with different affinities because they share a conserved N-terminal domain that determines the affinity for the DNA-binding region (Geanacopoulos and Adhya, 1997). In addition to the different promoter architectures, the self-regulatory circuits are different for each of these regulatory systems: AraC is subject to both positive and negative self-regulation; GutM seems to have dual regulation, positive self-regulation and negative regulation by a different specific regulator, and GalS is repressed both by itself and by and related apparently constitutive repressor. All of this stresses the necessity of additional experiments to determine whether promoter or regulatory architectures are capable of explaining the observed promoter activities.

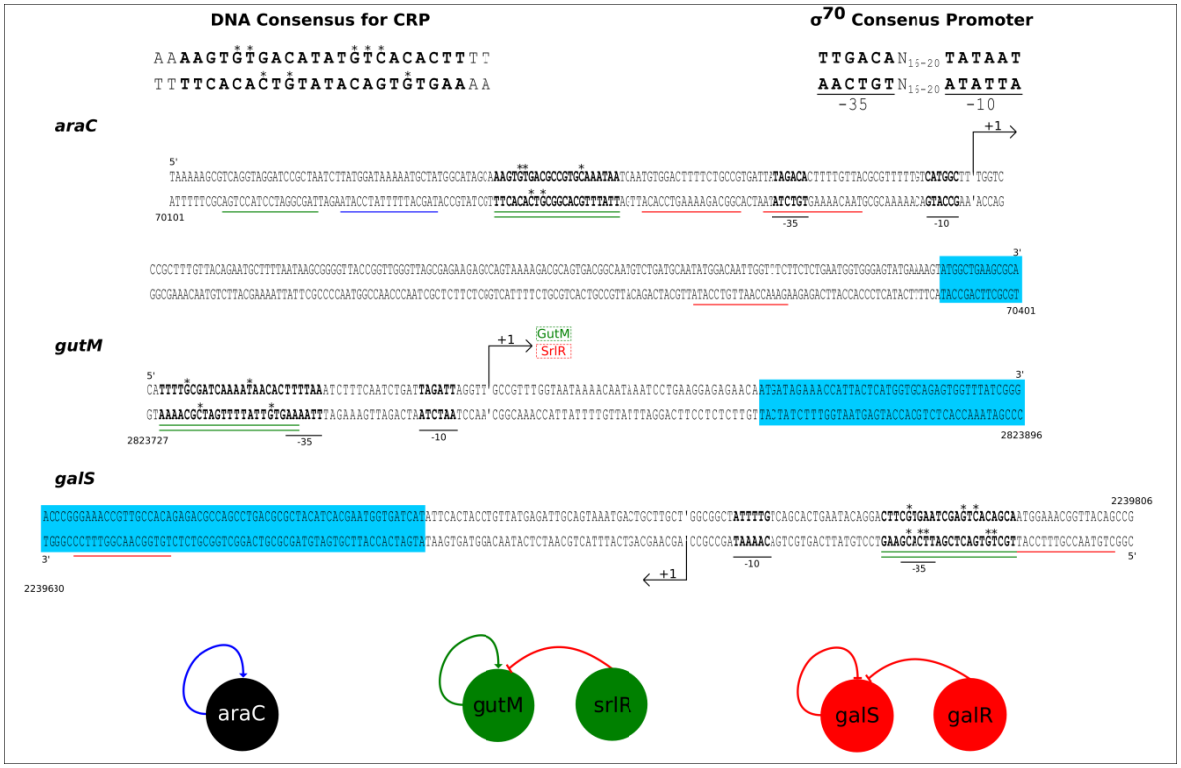

Fig. 7 Binding sites of CRP and σ70 in the studied promoters. The consensus binding sites for CRP and σ70 are show. Nucleotides marked with asterisks in the consensus for CRP are those that interact with the CRP protein (Parkinson et al., 1996). Numbers at the end of each sequence denotes genome positions of promoters. The initial translated amino acids of all regulatory proteins are shown in cyan. The DNA self-regulatory binding sites for each transcription factor are represented with single underlines. Double underlines are employed to denote the DNA binding sites for CRP. The -10 and -35 elements where the σ^{70}subunit of RNA polymerase binds are also marked. Colored underlines denote the type of regulation: green for activation, red for repression and blue for dual regulation. Finally, simplified schemes of regulatory switches for promoters are show.

4 Conclusions

It has been known for a long time that *E. coli* preferably consumes glucose over other carbon sources. However, we lack a complete knowledge of how this is achieved and regulated at the molecular level. In this work we present a proof of principle that permits to track the hierarchical use of carbon sources by following bacterial growth and promoter activities of the regulatory proteins that respond to specific sugars. We were able to identify the following order for the preferential use of carbon sources by *E. coli*: glucose > arabinose > sorbitol > galactose. A detailed analysis of regulator promoters for the corresponding catabolic operons indicates that this behavior can be due to at least three factors: 1) the coordinated activation of local regulators by the global regulator CRP, 2) the asymmetrical responses of transcription factors for specific and unspecific sugars and, 3) the architecture of promoters and operon-regulatory circuits. However, many questions remain open regarding the control mechanisms leading to this hierarchical behavior. Answering them will require a large amount of both experimental and mathematical modeling work. Finally, *E. coli* can consume more carbon sources than the ones here studied. It is still pending to test them to have a more complete scheme regarding the preferential use of carbon source in this bacterium.

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