molecular systems biology

# Shared control of gene expression in bacteria by transcription factors and global physiology of the cell

Sara Berthoumieux, Hidde de Jong, Guillaume Baptist, Corinne Pinel, Caroline Ranquet, Delphine Ropers, Johannes Geiselmann

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**Review timeline:**

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| Submission date: | 06 July 2012 |
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| Accepted: | 08 December 2012 |

## Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 17 August 2012

Thank you again for submitting your work to Molecular Systems Biology. We have now heard back from the three referees who agreed to evaluate your manuscript. As you will see from the reports below, the referees find the topic of your study of potential interest. They raise, however, substantial concerns on your work, which, I am afraid to say, preclude its publication in its present form.

While generally supportive, the reviewers felt that important issues needed to be addressed convincingly before this work would be appropriate for publication. One key issue was the suitability of the PRM promoter as an unbiased reporter of unregulated gene expression. The first two reviewers both felt other presumably constitutively active promoters should be tested experimentally and shown to produce similar results to this promoter, to support the robustness of these results. The first reviewer also felt that would be necessary to directly test the idea that cAMP levels do not affect expression from the constitutive promoter, to ensure that cell state and gene specific are being reliably separated.

Molecular Systems Biology generally requires that authors provide the data underlying all key experiments as supplementary materials. To make these data more accessible to readers in the event of publication, we provide a new functionality that allows 'source data' to be directly associated with selected figure panels (e.g. <http://tinyurl.com/365zpej>). This sort of figure-associated data may be particularly appropriate for this work. Please see our Instructions of Authors for more details on preparation and formatting of figure source data (<http://www.nature.com/msb/authors/index.html#a3.4.3>).

In addition, I noted the comments from Reviewer #1 regarding the quality of the pdf document. I have confirmed the this pdf looks worse, and cannot be pasted from, in pdf viewers other than Acrobat Reader -- suggesting that there may be some software compatibility issues at play here. I encourage you to submit the source Word or LaTeX document with your revision, so that we can ensure that the resulting pdf file is easily viewable across different software. If you are using LaTeX

 

please make sure to include all supporting files needed to compile the document (in a single zip file, please). I have attached our most recent LaTeX template files, in case they may be of use to you.

If you feel you can satisfactorily deal with these points and those listed by the referees, you may wish to submit a revised version of your manuscript. Please attach a covering letter giving details of the way in which you have handled each of the points raised by the referees. A revised manuscript will be once again subject to review and you probably understand that we can give you no guarantee at this stage that the eventual outcome will be favorable.

Yours sincerely,

Andrew Hufton

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Andrew L Hufton. PhD
Editor - Molecular Systems Biology
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Referee reports

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Reviewer #1 (Remarks to the Author):

Review: Berthoumieux et al.

The authors examine the following questions: In the regulation of bacterial gene expression, what is the relative weight of gene-specific transcription factors, versus gene-non-specific factors, here grouped under the title "physiological state of the cell". In the case studied by the authors (and another one briefly discussed), the answer is that whole-cell factors are dominant, while gene-specific factors are surprisingly minor in effect.

The subject matter, as well as the research approach, which combines quantitative experiments with simple theoretical analysis, are very appropriate for MSB. The work appears well conceived and executed, and the manuscript clearly written. Important controls are performed, to rule out possible artifacts. A few matters need addressing:

1) One issue that needs a little more attention, I think, is the precise definition of "physiological state of the cell". Which factors fall under this heading and which ones do not? Specifically, how did the authors decide that cAMP is NOT such a "cell-state" factor? One way to substantiate their claim is to show that cAMP does not affect expression of "constitutive" promoters such as PRM. cAMP levels can be manipulated by adding to the medium (in the proper genetic background, see e.g. Kuhlman et al., PNAS 2007), and PRM level read. Perhaps the authors can think of another way of testing that. The key is to verify beyond doubt that the separation of "cell state" and "gene specific" parameters is correctly done.

2) On a similar note, did the authors verify that the expression pattern exhibited by PRM is reflected in other "constitutive" promoters? This test is required to validate the description of PRM as "unregulated", dependent only on "cell state", and moreover, to validate the actual existence of such class of promoters, which only depend on the global "cell state".

3) Finally, a technical point: The manuscript as I received was very inhospitable for reviewing. Font size and line spacing were much smaller than is customary, and not easy to read. In addition, the PDF format was such that text could not be copied and pasted into another document, making the writing of a review more challenging than needed. I would encourage the authors to think more of the reviewers, and the journal to enforce more uniform submission formats.

 

Reviewer #2 (Remarks to the Author):

This paper addresses the coupling of gene regulation and global changes in cellular physiology. The authors study the response of the acs gene and its regulators in E. coli in response to glucose exhaustion as a model case. By comparing the dynamics of protein synthesis from these genes with a constitutive gene, they seperate generic global responses from the specific control of these genes. They find that most of the variation they see arises from the global change that affects all genes and only very specific parts of the response, can be attributed to specific regulation, in particular cAMP-induced activation of the acs gene.

The topic addressed here is timely and important. The study addresses an issue that is often overlooked and makes a clear case for the importance of generic global effects due to the changes of the cell's physiological state. The approach taken here, comparison of the dynamics of genes of interest with a constitute control, is straightforward but beautiful. I would hope that it will be used more frequently in the future. The authors approach this topic very carefully; in particular I like how they take care of the issue of plasmid copy number.
This said, I also have this impression that the results obtained with their nice method are not as surprising as claimed here (e.g. rpoS is known to be regulated at the levels of translation and proteolysis, as acknowledged by the authors) and I have some issues with specific technical points, listed below.

In summary, I am quite enthusiastic about the methods introduced here, but somewhat less enthusiastic about the results obtained with them so far, but I would recommend to give the authors a chance to submit a revised manuscript.

Specific issues:

1) The justification for using the constitutive control case is essentially that its known regulators are absent as this is a phage promotor. However phage promoters have co-evolved with host factors, so there may be host regulators affecting these promoters. For example, some phage promoters are affectd by ppGpp (e.g. Potrykus et al J Biol Chem 2002).
A better argument would be that this promoter behaves the same as other promoters believed to be constitutive (in that case unknown regulators are less likely as they would have to be shared by both) or that this promoter displaye the same steady-state growth rate dependence as other constitutive promoters (e.g. Liang et al 1999 or Klumpp et al 2009).

2) Does ppGpp play a direct role for any of these genes. While a ppGpp effect would not invalidate the results, it would change their interpretation as the global physiological effects would not be fully generic, i.e., for constitutive expression, but for a global stress response.

3) The use of the term 'gene expression' in the sense of a quantitative observable should be avoided as it is ambiguous when changing growth rates are considered, where different such observables have different growth rate dependecies (e.g. on p.5 "peak of acs expression", better use 'acs synthesis rate')

4) It might be worth noting that the acs promoter behaves as a constitutive promoter before the transition (Fig 5C), but (due to the normalization used) with a smaller coefficient.

5) I would have liked to see p_2(t), the dyamics of protein synthesis due to regulators, plotted togther with the observed dynamics p(t) at least in some cases.

Reviewer #3 (Remarks to the Author):

Manuscript Reference: MSB-12-3896
Authors: Hidde De Jong, Sara Berthoumieux, Delphine Ropers, Guillaume Baptist, Corinne Pinel, Caroline Ranquet, and Johannes Geiselmann
Title: Shared control of gene expression in bacteria by transcription factors and the physiological state of the cell

The analysis described in this manuscript shed on new light on the old biological question, which is

 

gene expression regulation during transitions between physiological states. The authors' basic question is how bacterial cell continuously adjust gene expression in response to the environmental changes. Two factors involved in this regulation, global effects of the physiological state and specific effects of transcription factors. Previous works mainly focused on the latter target and relatively little attention to the former issues had been paid. To clear this, the authors chose glucose depletion condition as a transition state and have performed mathematical model-based approach to distinguish between two effects, global physiological states and transcription factors, using time-series measurements of promoter activities by GFP fluorescence. They analyze transcription factors, CRP and Fis, which regulate a large number of enzyme genes in central metabolism in response to the available carbon source in the environment, and RpoS, which is a master regulator of E. coli stress response. Also they analyzed acs gene, whose product converts acetate to acetyl-CoA and regulated its expression during transition state. This gene is strongly expressed in the absence of glucose and may be an excellent indictor of the transcriptional response of carbon metabolism during transition state. They also measured cAMP concentration, which function as a signaling molecule to regulate the binding activity of CRP to the target DNA sites as a transcription factor. They started to construct the simple mathematical model of promoter activity to analyze the relative contribution of TFs and the physiological state to the response of the E. coli regulatory network. They carefully performed mathematical transformation with reasonable assumptions for simplification. And experimental time-series measurements of variables in the model were performed using transcriptional GFP fusion strains of the target genes.
The results described here gave us a caution that we have to pay attention more carefully to very basic biological events, which has long been believed as already cleared obvious issue, such as transcriptional regulation in E. coli cell. I think this paper is a good example to shed on new light to the old question. The authors used pRM promoter as a good indicators of physiological changes of the cell, which is known as a promoter not regulated by any TFs other than CI and Cro of phage lambda, not present in the cell measured.
The authors group has long been making efforts to develop quantitative measurement using fluorescence and their measurement is quite reliable.
So, my opinion is that, it is quite beneficial for readers of Molecular Systems Biology after consideration for improvement about a couple of comments listed below.

1) "substraction" may be replaced to "subtraction".
2) Figure 1, two dashed lines from CRP-cAMP to crp gene showed opposite effects and something confusing for the readers. There are two binding sites at the promoter region of crp and one function as activator and another as repressor. To avoid confusion, description may be added to the figure legend
3) For real-time monitoring of gene expression in Fig. 2 and Material and methods section, has the normalization been performed for quantitative measurements using 96 well microtiter plate? I think there are position effects on cell growth using microtiter plate, such as faster growth at edge side wells and low aeration in central wells. Or these biases are within the error range?
4) in figure 5, for example, panel A and B, clear correlation exist except at low and high value of log(PRM/P0RM). If I understand correctly, the authors do not discussed about the correlation during exponential, higher log(PRM/P0RM) value, and stationary, with low value. Is there any interpretation or hypothesis the reason why they showed low correlation? Or is this out of target this time?
5) in the table S3, "SC101ori" should be changed to "pSC101ori".
6) in figure S5. black and blue are hard to distinguish.
7) typo "workw" in Acknowledgements.

 

# Response to Reviewers of "Shared control of gene expression in bacteria by transcription factors and global physiology of the cell" (MSB-12-3896)

Sara Berthoumieux, Hidde de Jong, Guillaume Baptist,
Corinne Pinel, Caroline Ranquet, Delphine Ropers, Johannes Geiselmann

December 5, 2012

We would like to thank the reviewers for their encouragements and constructive criticism, which have helped us to improve the manuscript. In summary, we made the following major changes:

1. We compare the activity of the constitutive pRM promoter with another constitutive promoter (ptet), cloned into the same reporter vector, to confirm that the observed variation of pRM activity is not (or very unlikely) due to unobserved regulatory factors;
2. We investigate the effect of varying doses of external cAMP on the activity of the pRM promoter, in an appropriate genetic background, to confirm that the read-out of the global physiological state by means of the pRM promoter is not (specifically) affected by cAMP;
3. We explain in more detail the novelty and biological interest of our results, for the particular network studied and in a general setting;
4. We clarify the terminology of the paper. In particular, we clearly define what we mean by "global physiological state" of the cell as compared to specific regulatory factors;
5. We discuss the role of ppGpp as a possible explanatory factor for the observed changes in promoter activity;
6. We add figure source files for all reporter gene, cAMP and qPCR data, both in the main text and in the Supplementary Information.

Below we respond to each of the reviewer comments in detail and we summarize the changes made to the manuscript. The reviewer comments are in italic, and our response in default font.

## 1 Reviewer 1

*The authors examine the following questions: In the regulation of bacterial gene expression, what is the relative weight of gene-specific transcription factors, versus gene-non-specific factors, here*

*grouped under the title "physiological state of the cell". In the case studied by the authors (and another one briefly discussed), the answer is that whole-cell factors are dominant, while gene-specific factors are surprisingly minor in effect.*

*The subject matter, as well as the research approach, which combines quantitative experiments with simple theoretical analysis, are very appropriate for MSB. The work appears well conceived and executed, and the manuscript clearly written. Important controls are performed, to rule out possible artifacts. A few matters need addressing:*

*1) One issue that needs a little more attention, I think, is the precise definition of "physiological state of the cell". Which factors fall under this heading and which ones do not? Specifically, how did the authors decide that cAMP is NOT such a "cell-state" factor? One way to substantiate their claim is to show that cAMP does not affect expression of "constitutive" promoters such as PRM. cAMP levels can be manipulated by adding to the medium (in the proper genetic background, see e.g. Kuhlman et al., PNAS 2007), and PRM level read. Perhaps the authors can think of another way of testing that. The key is to verify beyond doubt that the separation of "cell state" and "gene specific" parameters is correctly done.*

**Answer:** The global physiological state refers to cellular factors that have an impact on the expression of all genes, such as the concentration of (free) RNA polymerase and ribosomes, gene copy numbers, and the size of amino acid and nucleotide pools. In steady-state conditions, the global physiological state is usually characterized by the growth rate (1). When studying transitions between steady states, as in our manuscript, a convenient read-out of the global physiological state is the activity of a constitutive promoter.

We did not include cAMP in the global physiological state of the cell, since it does not directly affect the expression of all genes (only those whose promoter activity is under the control of Crp·cAMP). There may be some indirect effects on the global physiological state though, as changes in the cAMP concentration may lead to changes in metabolism (due to changes in the concentration of Crp·cAMP-controlled enzymes) and thus to (weak) changes in the global physiological state. Notice that these indirect effects can be measured in our approach by means of the constitutive promoter, thus allowing us to separate specific cAMP-controlled effects from global physiological effects for the genes in our network.

For the above approach to work, however, we need to ascertain that there is no specific effect of cAMP on transcription from the constitutive promoter. Given that there are no Crp binding sites in the pRM promoter region we assumed this to be the case in the initial submission. Following the suggestion of the reviewer, we did additional experimental tests. Specifically, we transformed a $\Delta cya$ strain with the pZE1RM-*gfp* reporter plasmid and varied the external cAMP concentration within the appropriate range (see Fig. 1B of the article by Kuhlman *et al.* (7)). The growth kinetics of the strain and the observed activity of the pRM promoter did not significantly vary over a range of external cAMP concentrations in our conditions. This confirms that cAMP and the global physiological state, as measured by the activity of a constitutive promoter, can be

treated as independent inputs of the circuit.

**Action taken:** We state more clearly in the Introduction of the manuscript (p3, 2nd par) what we understand by global physiological state, we discuss the results of the experimental tests of the cAMP-independence of the pRM promoter in the Results section ("Monitoring the dynamic response...", p5, 5th par), and we provide details on the strains in Section S1 and on the experiments in the new Section S5, respectively.

*2) On a similar note, did the authors verify that the expression pattern exhibited by PRM is reflected in other "constitutive" promoters? This test is required to validate the description of PRM as "unregulated", dependent only on "cell state", and moreover, to validate the actual existence of such class of promoters, which only depend on the global "cell state".*

**Answer:** This is a good point raised by reviewer 2 as well. In order to test that pRM is an appropriate choice for a constitutive promoter, we cloned the ptet promoter (9) into the same plasmid backbone. ptet is believed to be constitutively transcribed in an *E. coli* wild-type strain (6). The measured time-varying activities of the pRM and ptet promoters were found to agree well both qualitatively and quantitatively in our conditions, suggesting that pRM indeed behaves as a constitutive promoter.[1]

**Action taken:** We discuss the results of comparing the pRM promoter with another constitutive promoter in the Results section ("Monitoring the dynamic response...", p5, 5th par), and we provide details on the construction and the experiments in Section S1 and the new Section S4, respectively.

*3) Finally, a technical point: The manuscript as I received was very inhospitable for reviewing. Font size and line spacing were much smaller than is customary, and not easy to read. In addition, the PDF format was such that text could not be copied and pasted into another document, making the writing of a review more challenging than needed. I would encourage the authors to think more of the reviewers, and the journal to enforce more uniform submission formats.*

**Answer:** We apologize for the manuscript quality. We used the Latex style file of the journal, but probably an older version and/or on a software platform with incompatibility issues.

**Action taken:** We used the latest version of the Latex style file provided by the editor, we increased line spacing, and we checked that the text can be copied from the file into different pdf readers.

[1] We also tried to construct reporters for the constitutive pL and pbla promoters used by Liang *et al.* (8), but failed to achieved this.

## 2 Reviewer 2

*This paper addresses the coupling of gene regulation and global changes in cellular physiology. The authors study the response of the acs gene and its regulators in E. coli in response to glucose exhaustion as a model case. By comparing the dynamics of protein synthesis from these genes with a constitutive gene, they seperate generic global responses from the specific control of these genes. They find that most of the variation they see arises from the global change that affects all genes and only very specific parts of the response, can be attributed to specific regulation, in particular cAMP-induced activation of the acs gene.*

*The topic addressed here is timely and important. The study addresses an issue that is often overlooked and makes a clear case for the importance of generic global effects due to the changes of the cell's physiological state. The approach taken here, comparison of the dynamics of genes of interest with a constitute control, is straightforward but beautiful. I would hope that it will be used more frequently in the future. The authors approach this topic very carefully; in particular I like how they take care of the issue of plasmid copy number. This said, I also have this impression that the results obtained with their nice method are not as surprising as claimed here (e.g. rpoS is known to be regulated at the levels of translation and proteolysis, as acknowledged by the authors) and I have some issues with specific technical points, listed below.*

*In summary, I am quite enthusiastic about the methods introduced here, but somewhat less enthusiastic about the results obtained with them so far, but I would recommend to give the authors a chance to submit a revised manuscript.*

**Answer:** The comments of the reviewer encourage us to explain more clearly and explicitly the novelty of our results from a biological point of view. Contrary to what may have been suggested in the manuscript, this does not concern so much the finding that global physiological effects dominate *rpoS* transcription. We believe the main novelty of our results lies elsewhere.

First, it is well-known that Fis and Crp are among the most pleiotropic transcription factors of the cell, located at the very top of the hierarchically-structured transcription regulation network in *E. coli*. Earlier work has shown that they are involved in a dense pattern of autoregulatory and cross-regulatory interactions, most notably involving a cross-inhibition motif (Fig. 1). The occurrence of this motif in our circuit suggests that it may behave as a bistable switch, along the lines of what has been shown before for synthetic circuits in bacteria and gene networks underlying developmental processes in higher organisms (3; 4; 11; 18). It notably leads to the hypothesis that the adaptation of gene expression in response to glucose depletion involves a regulatory master switch under the control of cAMP (17). In fact, the work reported in this manuscript originally started out as an attempt to test this hypothesis experimentally. The data eventually convinced us, however, that the dense pattern of interactions between Fis and Crp is not operative in physiological conditions where one would expect the switch to occur. Rather, the driving force behind the adaptation of gene expression turned out to be a factor that usually does not even figure in regulatory network diagrams. This was quite surprising to us (and it

took us some time to realize the importance of the phenomenon and come up with the proposed explanation)!

Second, generalizing beyond this particular circuit, the above finding goes against much of the received knowledge about the functioning of regulatory networks. As the reviewer also remarks, there is a tendency in (systems) biology to emphasize interactions involving transcription factors and other specific regulators. Inspired by our results and classical work in bacterial physiology, we propose that it may be more appropriate to invert this received view, and attribute the leading role to global physiological effects and a supporting role only to specific regulators. We are not the only authors to have come to these conclusions, as indicated in the discussion of previous work in the Introduction. However, to our knowledge, we are the first to explore this view dynamically, during a transition between growth states, and on the level of a small, but physiologically important network.

We recognize that the above arguments were very much implicit in the original submission.

**Action taken:** We better explain the biological interest and novelty of the results for the circuit studied in this manuscript and beyond, along the lines of the above answer, in the Introduction (p4, 2nd par) and in the Discussion section (p10, 2nd par). We also shorten and clarify the discussion of the results on *rpoS* (in the section "Validation of predicted...", p9, 2nd par) and remove *rpoS* from Fig. 1 to focus on the cross-inhibition network.

*Specific issues:*

*1) The justification for using the constitutive control case is essentially that its known regulators are absent as this is a phage promotor. However phage promoters have co-evolved with host factors, so there may be host regulators affecting these promoters. For example, some phage promoters are affectd by ppGpp (e.g. Potrykus et al J Biol Chem 2002). A better argument would be that this promoter behaves the same as other promoters believed to be constitutive (in that case unknown regulators are less likely as they would have to be shared by both) or that this promoter displaye the same steady-state growth rate dependence as other constitutive promoters (e.g. Liang et al 1999 or Klumpp et al 2009).*

**Answer:** The reviewer is right that the expression of some phage promoters is regulated by ppGpp (15). However, nothing is known about any ppGpp-dependence of pRM. In order to test whether pRM is an appropriate choice for a constitutive promoter, we followed the suggestion of this reviewer and reviewer 1 above. We cloned another promoter believed to be constitutive, the ptet promoter (6; 9), into the same plasmid backbone as pRM. The measured time-varying activities of the two promoters were found to agree very well, both qualitatively and quantitatively, in our conditions. We therefore conclude that pRM indeed behaves as a constitutive promoter.

**Action taken:** We compare the activity of the pRM promoter with another constitutive promoter in the Results section ("Monitoring the dynamics...", p5, 5th par), and we provide de-

tails on the construction and the experiments in Section S1 and in the new Section S4, respectively.

*2) Does ppGpp play a direct role for any of these genes. While a ppGpp effect would not invalidate the results, it would change their interpretation as the global physiological effects would not be fully generic, i.e., for constitutive expression, but for a global stress response.*

**Answer:** The transcription of some of the genes in the network of Fig. 1 is indeed under stringent control, and there is strong evidence that the *fis* promoter is directly regulated by (p)ppGpp (10; 12; 5; 20). It is not likely, however, that the observed dynamics of the promoter activities of the genes in our network can be accounted for by a dominant direct ppGpp effect, for the following two reasons. (i) It would require that ppGpp has exactly the same specific regulatory effect, qualitatively and quantitatively, on the *crp*, *fis*, and *rpoS* promoters, given the good quantitative correlation of the time-varying activities of these promoters. There is *a priori* no compelling reason for this to be the case. (ii) It would require transcription from the pRM and ptet promoters to be controlled by ppGpp as well, given the good quantitative correspondence of the activities of the above-mentioned network promoters and the two constitutive promoters. We concluded above that this is unlikely to be the case, so we attribute the observed variations in promoter activity to changes in the global physiological state of the cell.

Notice that the absence of a (strong) specific effect of (p)ppGpp on the promoters, at least in our conditions, does not contradict stringent control of the genes in the network. (p)ppGpp is a major factor in the control of the global physiological state (14; 19). It inhibits transcription of the rRNA operons, activates amino acid biosynthesis promoters, and indirectly influences the availability of free RNA polymerase by inhibiting strong $\sigma^{70}$ promoters (2; 13), thus influencing the activity of the gene expression machinery. Through these mechanisms, (p)ppGpp may have an indirect effect on the expression of a large number of genes.

**Action taken:** We extended the Discussion section of the manuscript with a paragraph on possible direct and indirect effects of ppGpp (p 9-10).

*3) The use of the term 'gene expression' in the sense of a quantitative observable should be avoided as it is ambiguous when changing growth rates are considered, where different such observables have different growth rate dependecies (e.g. on p.5 "peak of acs expression", better use 'acs synthesis rate')*

**Answer:** The reviewer is right that the use of "gene expression" in this sense is ambiguous. We indeed measure the (time-varying) synthesis rate of a reporter protein. Following the established terminology (*e.g.*, (16)), we refer to this observable as (promoter) activity (see the discussion in Sec. S2).

**Action taken:** We changed the terminology where appropriate, using 'gene expression' only when referring to the process (and not to an observable quantity).

*4) It might be worth noting that the acs promoter behaves as a constitutive promoter before the transition (Fig 5C), but (due to the normalization used) with a smaller coefficient.*

**Action taken:** Done, in the Results section of the manuscript ("Shared control of gene expression...", p8).

*5) I would have liked to see $p_2(t)$, the dyamics of protein synthesis due to regulators, plotted togther with the observed dynamics p(t) at least in some cases.*

**Action taken:** Done, in the new Fig. 6 of the manuscript. We discuss this figure in the Results section of the main text ("Shared control of gene expression...", p8, 1st par).

# 3 Reviewer 3

*The analysis described in this manuscript shed on new light on the old biological question, which is gene expression regulation during transitions between physiological states. The authors' basic question is how bacterial cell continuously adjust gene expression in response to the environmental changes. Two factors involved in this regulation, global effects of the physiological state and specific effects of transcription factors. Previous works mainly focused on the latter target and relatively little attention to the former issues had been paid. To clear this, the authors chose glucose depletion condition as a transition state and have performed mathematical model-based approach to distinguish between two effects, global physiological states and transcription factors, using time-series measurements of promoter activities by GFP fluorescence. They analyze transcription factors, CRP and Fis, which regulate a large number of enzyme genes in central metabolism in response to the available carbon source in the environment, and RpoS, which is a master regulator of E. coli stress response. Also they analyzed acs gene, whose product converts acetate to acetyl-CoA and regulated its expression during transition state. This gene is strongly expressed in the absence of glucose and may be an excellent indictor of the transcriptional response of carbon metabolism during transition state. They also measured cAMP concentration, which function as a signaling molecule to regulate the binding activity of CRP to the target DNA sites as a transcription factor.*

*They started to construct the simple mathematical model of promoter activity to analyze the relative contribution of TFs and the physiological state to the response of the E. coli regulatory network. They carefully performed mathematical transformation with reasonable assumptions for simplification. And experimental time-series measurements of variables in the model were performed using transcriptional GFP fusion strains of the target genes. The results described here gave us a caution that we have to pay attention more carefully to very basic biological events, which has long been believed as already cleared obvious issue, such as transcriptional regulation in E. coli cell. I think this paper is a good example to shed on new light to the old question. The authors*

*used pRM promoter as a good indicators of physiological changes of the cell, which is known as a promoter not regulated by any TFs other than CI and Cro of phage lambda, not present in the cell measured.*

*The authors group has long been making efforts to develop quantitative measurement using fluorescence and their measurement is quite reliable. So, my opinion is that, it is quite beneficial for readers of Molecular Systems Biology after consideration for improvement about a couple of comments listed below.*

*1) "substraction" may be replaced to "subtraction".*

**Action taken:** Done.

*2) Figure 1, two dashed lines from CRP-cAMP to crp gene showed opposite effects and something confusing for the readers. There are two binding sites at the promoter region of crp and one function as activator and another as repressor. To avoid confusion, description may be added to the figure legend?*

**Action taken:** Done.

*3) For real-time monitoring of gene expression in Fig. 2 and Material and methods section, has the normalization been performed for quantitative measurements using 96 well microtiter plate? I think there are position effects on cell growth using microtiter plate, such as faster growth at edge side wells and low aeration in central wells. Or these biases are within the error range?*

**Answer:** The data in Fig. 4 (and the other reporter gene measurements in Section S9) have not been normalized for growth differences between wells, except for synchronization of the curves to correct for small inoculation differences between wells (as explained in Section S2). The reviewer correctly observes that growth differences may occur between wells located on the edge and in the middle of the microplate. Edge wells were only included in the analysis when they did not exhibit any manifest growth difference as compared to the other wells.

**Action taken:** We added a phrase to Section S2 (p6) to explain this.

*4) in figure 5, for example, panel A and B, clear correlation exist except at low and high value of log(PRM/P0RM). If I understand correctly, the authors do not discussed about the correlation during exponential, higher log(PRM/P0RM) value, and stationary, with low value. Is there any interpretation or hypothesis the reason why they showed low correlation? Or is this out of target this time?*

**Answer:** The proposed promoter activity models capture global trends, but the data indeed seem to contain more subtle dynamical patterns, especially in the beginning and end of the ex-

periment. We think that these patterns arise from the following two (not necessarily exclusive) circumstances. (i) When the signal is close to the background (for small absorbances), the data analysis procedures (which involve taking derivatives of the background-corrected signals, see Section S2) become more difficult to apply. This usually leads to less precision and large confidence intervals. (ii) Some of the deviations, especially after the growth arrest, may be due to the finetuning effect of (other) specific regulators. We did not develop this point in the current manuscript (which would require the use of different types of models). We emphasize that deviations from the global trend remain small though, and are often not significant (have large confidence intervals). This is clearly seen for *fis* and *crp* in the figure which plots $p_2(t)/p_2^0$ over time (see Fig. 6 in the revised manuscript, added on request of reviewer 2).

**Action taken:** We discuss this issue in the Results section ("Shared control of gene expression...", p8, 1st par).

*5) in the table S3, "SC101ori" should be changed to "pSC101ori".*

**Action taken:** Done.

*6) in figure S5. black and blue are hard to distinguish.*

**Action taken:** Black was changed to green.

*7) typo "workw" in Acknowledgements.*

**Action taken:** Done.

# References


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