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Positive allosteric feedback regulation of the stringent response enzyme RelA by its product

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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 27 April 2012

Thank you for you patience while your study has been under peer-review at EMBO reports. We have now received the three enclosed reports on it; referee 1 is from the stringent response field and 2 and 3 work on allosteric enzymatic mechanisms. We have also extensively discussed the study after peer-review among the team -including our Chief Editor- and an expert editorial advisor, who knows the journal well.

Unfortunately, the outcome of all these discussions is not positive, as we have decided not to consider your manuscript further. In this case, EMBO reports was interested in the study for its claim of a new mode of enzyme regulation, as only in this light would the study provide the type of advance of general interest that we seek to publish. Taking this into consideration, the clear indication in referee 3's report that this is not the first such example limits the novelty and general interest of the findings, and precludes publication of the manuscript here. Thus, although this referee was initially supportive of the work, upon further discussion it became clear that his/her indication of lack of novelty is a strong argument against publication. Referee 2 considers that the study should be developed further and submitted as a full report elsewhere. From the point of view of the stringent response, the study reports two additional features that extend the Nierhaus hopping model and will be of interest to those working closer to the field.

As I mentioned above, this decision was made after further consultation with the referees, the editorial team (including the Chief Editor) and an editorial advisor. Overall, we do not doubt that your manuscript describes findings worth publishing -and people in the field will find them valuable- but they do not offer the sort of general conceptual advance that we look for in an EMBO reports study. We would suggest that you submit your work alongside these anonymous reports to a more specialized journal, where they are likely to be conducive to a positive answer in a timely manner.

I am sorry to disappoint you on this occasion and thank you for having considered EMBO reports for publication.

Yours sincerely,

Editor
EMBO Reports

REFEREE REPORTS:

Referee #1:

The hopping model of the Nierhaus group explained the fact that a small number of molecules of RelA could monitor the vast amount of ribosomes with a deacylated tRNA at the A site in the cell. The model received an extension by the authors in a previous publication showing that RelA activated by the ribosome leaves the ribosome keeping its activated state for a while. Here the authors report two additional features of the stringent response: (i) The product (p)ppGpp stimulates the RelA activity, and (ii) isolated L11 known to be an essential ribosomal component for mediating the RelA activation can stimulate the RelA activity in the absence of 70S particularly in the presence of (p)ppGpp. These are interesting findings that broaden our knowledge about the regulation of the stringent response.

Minor points:
1: page 3, paragraph 2, line 1: One does not add 100 µM to a system, rather one adds (p)ppGpp yielding a concentration of 100 µM.
2: page 3, paragraph 2, second last line from the bottom: read (<B>Fig. 1b</B>, black solid circles).

Referee #2:

I am no fan of papers whose existence seems to depend upon claims of "novelty", or of being "first". If such superfluous claims and discussion were removed from this paper, there would be room for important information that would increase the reader's confidence in the competence and relevant knowledge of the authors. The authors say that their discovery with RelA, provids the first case of direct positive enzyme regulation by its product. Oxygen binding by hemoglobin seems close to being a counterexample. With enough qualifiers, anything can be a first.

Similarly, I dislike author-invented paper-specific abbreviations, as they make reading some papers into the equivalent of a vocabulary lesson in a foreign language. In this case, just one was used, SR. It was used four times, which saves less than a line of text. Why should I have to learn what special abbreviations mean, I need to focus on the science.

Lots of readers will be confused by the fact that ppGpp was being synthesized instead of pppGpp (because GDP was used as a substrate instead of GTP). A simple sentence would have prevented such confusion.

If the product of the relA catalyzed reaction is also to be an allosteric effector, relA almost has to be an oligomer. In fact it is. The authors should have foreseen this concern and explicitly said that relA

is a dimer.

The paper claims that the in vivo concentration of relA is about 30 nM, which seems awfully low. This is about 30 molecules per cell. The turnover number they report for relA is about 40/min. These numbers lead to more than an hour being required in vivo in order for ppGpp levels to be able to reach the 100 mM concentrations being used to "allosterically" regulate relA. The in vivo response time to amino acid starvation is much less than 50 minutes, however, and thus at least one of the numbers seems to be incorrect.

I am not highly knowledgeable in the field of nucleotide metabolism, but I have the nagging fear that some sort of extraneous exchange reaction might be misleading the authors. I urge the authors and editor to give consideration to publishing the current finding in a complete and fully documented way that will convince everyone of the validity of the positive allosteric effect.

Referee #3:

This work is interesting, well carried out and well described. The proposed regulatory function of product activation is convincing.

However, there are some problems with the paragraph beginning "Until now..." on p. 6. One cannot use a book published in 1993 (19 years ago) as evidence for what has been true "until now", especially when the book in question was a facsimile reprint (with no revision) of one published in 1975 (37 years ago). If the authors wish to maintain this sentence they need to justify it by reference to more recent work.

In the next sentence, it is too strong to say that "negative feedback auto-inhibition ... guarantees homeostasis". It contributes to it, certainly, but it's not so easy to guarantee anything.

In the next sentence, the authors claim that this is "the first documented case of positive regulation by the product acting via a direct allosteric mechanism". That is again too strong. Product activation is certainly unusual, but it is not unprecedented, and the paper should refer to work on lipoamide dehydrogenase (Biochim. Biophys. Acta 48, 33-47 (1961)) and nitrite reductase (Biochem. J. 175, 495-499 (1978)). The authors should check whether there are more recent examples.

Correspondence - authors' appeal 18 May 2012

Thank you for your careful consideration of our manuscript EMBOR-2012-35998-T. It is clear from the referee comments and from your letter that there is no critique of our experimental methods or any doubt about our conclusions, apart from a vague and, as we can see, science-unrelated 'nagging fear' of the second referee. The only reason for the rejection appears to be the concern that our results are not novel or important enough for publication in EMBO Reports. We strongly disagree with the claim of lack of novelty, and consider our results to be of considerable importance, an opinion held not just by us, but also by also by prominent researchers in the fields of translation (Professor Wilson, letter of support attached) and stringent response (Professor Cashel, the co-discoverer of the ppGpp alarmone, has, I believe, contacted you directly). Therefore, we would like to appeal the rejection. Our appeal is set out in detail below.

First, we do believe that our results are important and novel for the whole field of enzymology. Both referee 1 and referee 3 have provided examples of positive allosteric regulation of an enzyme by its product. Referee 1 suggested haemoglobin and oxygen, even though haemoglobin is not an enzyme and oxygen is not its product. Referee 3, in contrast, made the valid point that two types of enzymes are indeed activated in the presence of their product, NAD+ (1,2). There is, however, a fundamental difference between activation of RelA by ppGpp and the activation scenarios in these two examples (1,2). Both these enzymes are activated by NAD+ via alterations in the oxidative environment, and not via direct allosteric regulation. The RelA product, ppGpp, certainly does not activate RelA via an oxidation pathway. Furthermore, ppGpp is known to be an allosteric regulator of enzymes other than RelA, with RNA polymerase as a striking example (3,4). To our knowledge there are no

examples of allosteric regulation of an enzyme by its product in any of the enzymology textbooks we have scrutinized (5-8). Nor could we find any examples using PubMed of allosteric product regulation of an enzyme, suggesting that we have discovered an entirely new phenomenon. Therefore our statement that we report a novel mechanism of considerable importance for the whole enzymology community has not been falsified by the referees.

Second, we would like to stress that RelA is not just yet another enzyme; it is the hub of a vast, global regulatory network that senses the translational state of the ribosome and synthesizes ppGpp in response to that state. ppGpp in turn regulates production of ribosomes, therefore acting as a master regulator of the most energy-consuming intracellular process (9,10). RelA-mediated production of ppGpp not only regulates the stringent response to stress stimuli, such as amino acid starvation or heat shock, but also the adaptation of bacteria to environmental changes (11), cell cycle (12), and tolerance to antimicrobials (13), and this mechanism is nearly universal in all bacteria (14). This makes our results important to a broad scientific community including medicine, microbiology, molecular biology, biochemistry, systems biology and evolutionary biology.

Our demonstration of RelA activation by ppGpp is much more significant than merely adding another 'feature' to the 'Nierhaus hopping model' (although our own observations in a previous paper suggested an 'extended hopping model' of RelA (15)). Perhaps in writing for the short report format we failed to spell out the implications of our results clearly enough. In fact, ppGpp-mediated cross-talk is not limited to RelA molecules. Our recent in silico investigations demonstrated that there are about 30 different groups of RelA-SpoT Homologue (RSH) proteins distributed diversely among bacteria (14). Our results will prompt investigations of the cross-talk among these groups, addressing questions such as which RSH enzymes are ppGpp-sensitive, which are not, and what is the computational logic behind the RSH systems. Clearly, an alarmone system that senses and reacts to its output using direct regulation of the enzymatic activity is a unique feature of the stringent response system. Hence, the regulation of such product-regulated enzymatic hub will be of high interest to the systems biology community.

For the reasons given above, we urge you to reconsider your decision on our manuscript. We feel that this paper will receive much attention, particularly in the enzymology community (rare enzymatic regulation) as well as in the field of systems biology (for its novel regulatory pathway of a key hub-enzyme), and general and medical microbiology. After the oral presentation of our data at the Microbial Stress 2012 conference I was invited by a major pharmaceutical company to present the biotechnological applications of our results, which clearly suggests importance for the field of applied science as well. Moreover, Dr. Catherine Goodman, an editor for Nature Chemical Biology, expressed her intention to cover our results as a research highlight article.

Due to its broad appeal and inherent citability we feel it is well-suited for publication in EMBO Reports. We are ready to resubmit a modified version of the manuscript along with point-by-point answers to the referees in a response letter.

Best regards,
On behalf of all the authors

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1. Coleman, K.J., Cornish-Bowden, A. and Cole, J.A. (1978) Activation of nitrite reductase from Escherichia coli K12 by oxidized nicotinamide-adenine dinucleotide. Biochem J, 175, 495-499.
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15. English, B.P., Hauryliuk, V., Sanamrad, A., Tankov, S., Dekker, N.H. and Elf, J. (2011) Single-molecule investigations of the stringent response machinery in living bacterial cells. Proc Natl Acad Sci U S A, 108, E365-373.

2nd Editorial Decision 24 May 2012

I have now heard back from referees two and three, with whom I consulted regarding your letter of appeal. They both had further comments, which you will see below. Given these discussions, we have decided to give you the opportunity to revise your study.

Please note that we cannot ultimately accept your study for publication unless it receives the referees' support. It is clear that they take issue in the way the work is portrayed, as well as raise other concerns in both their initial reports and their comments below. All referee concerns would have to be addressed during revision, and the referees would also ultimately have to be convinced of the novelty of the proposed mechanism of enzyme activation. Please note that it is EMBO reports policy to undergo one round of revision only and, thus, acceptance of your study will depend on the outcome of the next, final round of peer-review.

Yours sincerely,

Editor
EMBO Reports

Referee comments:

Referee 2:

I am sorry, I do not have extensive knowledge of the literature on allosteric enzymes, and therefore, I cannot confirm that this is the FIRST allosteric enzyme that is positively regulated by the enzyme's product.

However, he authors' response raises concerns. They ignore the numerical discrepancy that I previously noted. The discrepancy invalidates the biological significance of their findings and all that they write about their significance. To me this is more important than whether or not the prior examples of positive regulation are exactly what that authors had in mind in their definition of firstness.

In summary, I have scientific concerns with the paper. I would not allow any of my students to submit such work to any journal.

Referee 3:

Given the author's arguments regarding the novelty of the enzymatic regulation, there is no reason why a revision should not be considered.

However, the list of references they provide for researching the novelty of allosteric activation of an enzyme by its product is worrying, as it suggests that the authors made no serious attempt to find out what was known already when they wrote their paper.

Ref. 4 doesn't seem relevant to the point I was making, so I'm not sure why it is listed. Of the others, the most recent is from 12 years ago: in no way can it be used to justify a statement that begins "until now". Now is the year 2012. In any case, the reference in the paper is not to ref. 5, 6 or 7, but to ref. 8, which, as I have noted, dates from 1975, not 1993 as claimed. Surely the authors must have access to some recent sources, or if not they must surely have some better informed colleagues that they can ask.

In any case, I'm not in the least surprised if none of the above textbooks discuss product activation, as it is not the sort of thing any of the authors concerned are interested in. Even if they were they might not think it important enough to discuss, and, in fact, I do not know of any textbook that discusses it. But the authors cannot conclude from taking a brief look at a few textbooks and finding no mention of product activation that there are no examples. They need to do some serious reading of the primary literature.

My main concern was that they are making misleading and unsupported statements about prior knowledge: they would need to fix these (and take to heart the other referees' comments also).

1st Revision - authors' response 01 June 2012

Referee #1:

Reviewer's comment:

The hopping model of the Nierhaus group explained the fact that a small number of molecules of RelA could monitor the vast amount of ribosomes with a deacylated tRNA at the A site in the cell.

The model received an extension by the authors in a previous publication showing that RelA activated by the ribosome leaves the ribosome keeping its activated state for a while. Here the authors report two additional features of the stringent response: (i) The product (p)ppGpp stimulates the RelA activity, and (ii) isolated L11 known to be an essential ribosomal component for mediating the RelA activation can stimulate the RelA activity in the absence of 70S particularly in the presence of (p)ppGpp. These are interesting findings that broaden our knowledge about the regulation of the stringent response.

Minor points:
1: page 3, paragraph 2, line 1: One does not add 100 μM to a system, rather one adds (p)ppGpp yielding a concentration of 100 μM.

Our response: Amended accordingly.

Reviewer's comment:

2: page 3, paragraph 2, second last line from the bottom: read (<B>Fig. 1b</B>, black solid circles).

Our response: Amended accordingly.

Referee #2:

Reviewer's comment:

I am no fan of papers whose existence seems to depend upon claims of "novelty", or of being "first". If such superfluous claims and discussion were removed from this paper, there would be room for important information that would increase the reader's confidence in the competence and relevant knowledge of the authors. The authors say that their discovery with RelA, provids the first case of direct positive enzyme regulation by its product. Oxygen binding by hemoglobin seems close to being a counterexample. With enough qualifiers, anything can be a first.

Our response: Oxygen binding to hemoglobin is in fact not a counterexample: hemoglobin is not an enzyme, and oxygen is hardly its product. That said, we strongly object to the referee's assertion that our paper's 'existence seems to depend upon claims of "novelty", or of being "first"'. Our results are of very important for understanding the molecular mechanism of the stringent response and bacterial adaptation in general.

Reviewer's comment:

Similarly, I dislike author-invented paper-specific abbreviations, as they make reading some papers into the equivalent of a vocabulary lesson in a foreign language. In this case, just one was used, SR. It was used four times, which saves less than a line of text. Why should I have to learn what special abbreviations mean, I need to focus on the science.

Our response: We now use the full expression as per the referee's wishes. However, we would like to point out that we didn't invent SR as an abbreviation for the stringent response, it was coined more than twenty years ago (Gilbert et al., 1990), and is used occasionally since then (e. g. in the recent *Science* paper by Nguyen and colleagues (Nguyen et al., 2011)).

Reviewer's comment:

Lots of readers will be confused by the fact that ppGpp was being synthesized instead of pppGpp (because GDP was used as a substrate instead of GTP). A simple sentence would have prevented such confusion.

Our response: A clarifying sentence has been added, as suggested by the reviewer.

Reviewer's comment:
If the product of the relA catalyzed reaction is also to be an allosteric effector, relA almost has to be an oligomer. In fact it is. The authors should have foreseen this concern and explicitly said that relA is a dimer.

Our response: Allosteric regulation does not have to act via oligomerization: there are plenty of examples of both monomeric and oligomaric proteins being regulated allostrically (Kalodimos, 2011). RelA was shown to have an auto-inhibitory domain (Mechold et al., 2002), and allosteric regulation of proteins with auto-inhibitory domains usually is achieved on the intra-molecular level and does not require oligomerization (Kobe and Kemp, 1999). Therefore at this point it is possible to envision several mechanisms of ppGpp-mediated activation, and the question of oligomerisation remains open.

Since we do not provide structural insights into the mechanism, we prefer not to speculate on this point, especially given the strict length limitations imposed by the *EMBO Reports* format.

Reviewer's comment:

The paper claims that the in vivo concentration of relA is about 30 nM, which seems awfully low. This is about 30 molecules per cell. The turnover number they report for relA is about 40/min. These numbers lead to more than an hour being required in vivo in order for ppGpp levels to be able to reach the 100 mM concentrations being used to "allosterically" regulate relA. The in vivo response

time to amino acid starvation is much less than 50 minutes, however, and thus at least one of the numbers seems to be incorrect.

And from this reviewer's second letter:

However, he authors' response raises concerns. They ignore the numerical discrepancy that I previously noted. The discrepancy invalidates the biological significance of their findings and all that they write about their significance. To me this is more important than whether or not the prior examples of positive regulation are exactly what that authors had in mind in their definition of firstness.

In summary, I have scientific concerns with the paper. I would not allow any of my students to submit such work to any journal.

Our response: The *in vivo* relevance of our data is validated by the observation that RelA synthesis is dramatically activated by ppGpp concentrations in the low *in vivo* range (50 uM, Figure 1c), not by the turnover rates we obtain, which are quite similar to those reported in other *in vitro* investigations of RelA. Moreover, it should be noted that rates of protein synthesis *in vitro* systems are in general significantly lower then those estimated in the living cells, which, however, does not preclude *in vitro* experimentation being the main source of our current knowledge about translation. Nor does it render *qualitatively* new phenomena, like the ppGpp dependent stimulation of RelA synthesis of ppGpp, irrelevant.

As to the 'numeric discrepancy', one should note, that, first, the activity of the purified RelA protein is below 100%, and the artificial polyU *in vitro* system may not activate RelA as well as the stalled ribosomal complexes in the living cell. Second, the RelA turnover rate in our system is about 270/min at 0.5 uM polyU-programmed ribosomes in the presence of deacylated tRNA (Figure 1d). This is about seven times larger than the 40/min turnover rate that the referee refers to. Third, *in vivo*, the ribosome concentration is about 20 uM, and the difference in ribosome concentration between the *in vivo* and *in vitro* situations probably accounts for the discrepancy between the two turnover rates.

Reviewer's comment:

I am not highly knowledgeable in the field of nucleotide metabolism, but I have the nagging fear that some sort of extraneous exchange reaction might be misleading the authors. I urge the authors and editor to give consideration to publishing the current finding in a complete and fully documented way that will convince everyone of the validity of the positive allosteric effect.

Our response: It is hard to interpret the referee's statement 'I have the nagging fear…'. We take

this to mean that the referee believes that we have not investigated the putative role of artifacts in our novel results. We can, however, assure the referee that we have been able to eliminate numerous a priori possible reasons for the observed phenomena. We thoroughly address and reject all possible causes of artifacts in our Supplemental Information (see Supplementary Figures 1-9).

Referee #3:

Reviewer's comment:

This work is interesting, well carried out and well described. The proposed regulatory function of product activation is convincing.

However, there are some problems with the paragraph beginning "Until now..." on p. 6. One cannot use a book published in 1993 (19 years ago) as evidence for what has been true "until now", especially when the book in question was a facsimile reprint (with no revision) of one published in 1975 (37 years ago). If the authors wish to maintain this sentence they need to justify it by reference to more recent work.

Our response: Amended accordingly. In the revised version we cite references suggested by the referee (Coleman et al., 1978; Massey and Veeger, 1961) (see below). The abstract is also amended, to state that 'We show that ppGpp dramatically increases the turnover rate of ribosome-dependent ppGpp synthesis by RelA, resulting in direct positive enzyme regulation by its product.'

Reviewer's comment:

In the next sentence, it is too strong to say that "negative feedback auto-inhibition ... guarantees homeostasis". It contributes to it, certainly, but it's not so easy to guarantee anything.

Our response: In the revised version this statement is removed altogether.

Reviewer's comment:

In the next sentence, the authors claim that this is "the first documented case of positive regulation by the product acting via a direct allosteric mechanism". That is again too strong. Product activation is certainly unusual, but it is not unprecedented, and the paper should refer to work on lipoamide dehydrogenase (Biochim. Biophys. Acta 48, 33-47 (1961)) and nitrite reductase (Biochem. J. 175, 495-499 (1978)). The authors should check whether there are more recent examples.

And from this reviewer's second letter:

Given the author's arguments regarding the novelty of the enzymatic regulation, there is no reason why a revision should not be considered.

However, the list of references they provide for researching the novelty of allosteric activation of an enzyme by its product is worrying, as it suggests that the authors made no serious attempt to find out what was known already when they wrote their paper.

Ref. 4 doesn't seem relevant to the point I was making, so I'm not sure why it is listed. Of the others, the most recent is from 12 years ago: in no way can it be used to justify a statement that begins "until now". Now is the year 2012. In any case, the reference in the paper is not to ref. 5, 6 or 7, but to ref. 8, which, as I have noted, dates from 1975, not 1993 as claimed. Surely the authors must have access to some recent sources, or if not they must surely have some better informed colleagues that they can ask.

In any case, I'm not in the least surprised if none of the above textbooks discuss product activation, as it is not the sort of thing any of the authors concerned are interested in. Even if they were they might not think it important enough to discuss, and, in fact, I do not know of any textbook that discusses it. But the authors cannot conclude from taking a brief look at a few textbooks and finding no mention of product activation that there are no examples. They need to do some serious reading of the primary literature.

My main concern was that they are making misleading and unsupported statements about prior knowledge: they would need to fix these (and take to heart the other referees' comments also).

Our response: The revised version is considerably more balanced and cautions. We now cite the references suggested by the referee (Coleman et al., 1978; Massey and Veeger, 1961). There is, however, a critical difference between RelA activated by ppGpp and these two examples. Both of the enzymes suggested by the referee are activated by NAD+ via *oxidation*, not via *allosteric regulation*. RelA's product, ppGpp, is highly unlikely to act via this oxidation and is known to be an *allosteric* regulator of enzymes other then RelA (Kanjee et al., 2011; Reddy et al., 1995).

As we stated in the previous communication, to our knowledge there are no examples of *positive* allosteric regulation of enzyme by its product in general enzymology textbooks we could find, even though *all* of the abovementioned textbooks give examples of negative regulation by the product (Copeland, 2000; Fersht, 1998; Price and Stevens, 1999; Segel, 1993). Presumably, if product *inhibition* is important enough to be discussed in textbooks, product *activation* would be as well if such cases were reported in the literature. The absence of product activation in textbooks indicates to us that this is at least a very rare phenomenon of enzymatic regulation.

Our search of examples of positive allosteric regulation by product was by no means limited to consulting textbooks; we performed extensive PubMed and Google Scholar searches, and, just as the referee suggested, we did attempt to consult more knowledgeable colleagues. We have presented our data at numerous conferences, as an oral presentation (Microbial Stress (Belgirate 2012), Biophysical Society Meeting (San Fransisco 2010), Bacterial Cell Biology (Cancun 2010), FEBS congress (Göteborg 2010), Emerging Tools in Quantitative Fluorescence Microscopy for Systems Biology (Portugal 2011) and MPI (Marburg 2012)) and as a poster (Protein Synthesis and Translational Control (Heidelberg 2011)), as well as at departmental meetings in Uppsala University, University of Tartu, Albert Einstein College of Medicine, University of Strasbourg and Gene Center at the University of Munich. We specifically asked the audience to provide examples of positive allosteric regulation by product, and so far we did not get any. Several suggestions initially seemed promising, but did not withstand further scrutiny, such as activation of phosphofructokinase *1* by the product of phosphofructokinase *2*, F2,6BP (Sola-Penna et al., 2010) (the catch is that here we have a positive cross-talk between the two enzymes, rather then *auto*-activation), or allosteric activation of several guanine-nucleotide exchange factors (GEF) by their product, the GTPase:GTP complex (Butty et al., 2002; Margarit et al., 2003; Stalder et al., 2011) (the catch is that GEF is not an enzyme since it does not catalyze a *chemical* reaction).

We certainty cannot claim encyclopedic knowledge of the enzymology literature. However, our combined expertise in bacterial physiology and systems biology makes us pretty confident that this is the first example of a *regulatory* enzyme activated by the low-molecular mediator molecule it produces. Given high diversity of such systems in bacteria, where multiple nucleotide-based messengers regulate stress responses, virulence, biofilm formation and much more (Pesavento and Hengge, 2009), this alone makes our results very import.

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3rd Editorial Decision 06 June 2012

Thank you for the submission of your revised study to EMBO reports. I have now received feedback from referees 2 and 3 of your previous submission, both of whom support publication of the study

and have no further comments. I am therefore writing with an 'accept in principle' decision, which means that I will be able to accept your manuscript for publication once several issues/corrections have been addressed, as follows.

In general, please read our instructions to authors (a link to which can be found in our homepage) and ensure your study is appropriately formatted. Numerous issues have come up with respect to this:

- we do not allow the presentation of a supplementary discussion section, so this will have to be removed. Relevant content can be included in the main text, if space is available (please note that it must be a maximum of 27,500 characters including spaces)

- the main text must include a methods section that details those essential to the understanding of the experiments. More detailed explanations necessary to reproduce them may be presented as supplementary information, only if space is an issue.

- the majority of figures (both main and supplementary) in which statistical analysis has been performed lack all relevant information in the figure legend. Please ensure that all relevant figures and supplementary figures have been generated according to proper statistical analysis procedures (using data from at least three independent experiments and not replicates), and all figure legends include information on the number of independent experiments measured and the type of error bars used. For guidance, please refer to Cumming et al. JCB 2007.

- we limit the presentation of supplementary figures to those strictly necessary and all must be directly related to a main figure. Main results may no be presented as supplementary, only controls, replicates in similar conditions, etc. In this regard, it seems excessive to have 9 supplementary figures for a 2 figure study. You could include up to two more in the main text, if they are of sufficient relevance. In addition, related supplementary figures could be combined into one with several panels (for example, all the ones relevant to figure 1 as one figure and the ones pertaining to figure 2 as another).

If all remaining corrections have been attended to, you will then receive an official decision letter from the journal accepting your manuscript for publication in the next available issue of EMBO reports. This letter will also include details of the further steps you need to take for the prompt inclusion of your manuscript in our next available issue.

Thank you for your contribution to EMBO reports.

Yours sincerely,

Editor
EMBO Reports

4th Editorial Decision 02 July 2012

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports. All remaining issues have been addressed and corrected in the final version accordingly.

Thank you for your contribution to EMBO reports and congratulations on a successful publication. Please consider us again in the future for your most exciting work.

Yours sincerely,

Editorial Assistant
EMBO Reports

