EMBO reports

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Organismal regulation of XBP-1-mediated unfolded protein response during development and immune activation

Jingru Sun, Yiyong Liu and Alejandro Aballay

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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 11 April 2012

Thank you for your submission to EMBO reports. First of all, please accept my apologies for the time it has taken us to contact you with a decision on your study, as we have only now received a full set of referee reports (which are enclosed). Referee 1 took part in a structured referee report trial and so this report is in a different format. As you will see, although all the referees find the topic of interest and referees 1 and 3 are more positive about the study, referee 2 considers it is still preliminary and should be developed substantially before it becomes appropriate for publication here.

All of them request a number of technical improvements of the data, and further experiments that are needed to develop your story sufficiently for EMBO reports. Given that all referees provide constructive suggestions on how to strengthen the study, I would like to give you the opportunity to revise your manuscript. In this case, we consider important that all referee concerns be addressed in full during revision. If the referee concerns can be adequately addressed (and they support the current message of the study), we would be happy to consider your manuscript for publication. However, 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.

I look forward to seeing a revised form of your manuscript when it is ready. In the meantime, do not hesitate to get in touch with me if I can be of any assistance.

Yours sincerely,

Editor
EMBO Reports

REFEREE REPORTS:

Referee #1:

1. Do the contents of this manuscript report a single key finding? YES / NO

Yes, the manuscript "Temporal and organismal regulation of XBP-1-mediated unfolded protein response" by Jingru Sun and colleagues, determines the effects of mutant octr-1 in C. elegans on the regulation of UPR pathway that includes the transcription factor XBP-1. They describe this regulation at the temporal level because they show the splicing of XBP-1 only at adult stage not during development, and this correlates with the increased levels of target genes like hsp-4 and Y41C4A.11.

2. Is the main message supported by compelling experimental evidence? YES / NO

No, overall this is a well-done study that provides new and useful information about the regulation of splicing of XBP-1 on the development of C. elegans by octr-1; however, additional experiments and controls will be useful to explain the phenomena.

1.- The authors compared by qPCRs levels of targets genes that are dependent and independent of XBP-1 and its splicing, on mutants of octr-1 at basal levels and stimulated with P. aeruginosa, at two different stages of development. It is necessary to see if the genes showed in 1A and 1B changes and they have to include the splicing of XBP-1 at the same stages.

2.- Images 1B and 1E are over-exposed to be quantified, and the quantification on 1C and 1F should shown as the ratio of spliced XBP1/ total XBP1, being total XBP1 the addition of spliced XBP1 plus unspliced XBP1. Also, they should include control samples: wt and octr-1 at basal levels.

3.-On figure 2 and 3, showing only one animal per stage of development is not enough to depict the phenomena, and it is necessary include what is the criteria of selection of ROI on Figure 2 and Figure 3.

4.- Figure 2 and 3 can be evaluated the expression of GFP by Western Blot to validated the results obtained by fluorescence intensity.

3. Have similar findings been reported elsewhere (e.g. on a closely related protein; in another organism or context)? YES / NO

No.

4. Is the main finding of general interest to molecular biologists? YES / NO

Yes, it determined the regulation on the development process related to XBP-1. It has been described XBP-1 is necessary for the homeostasis, development of immune system and secretory organs.

5. After appropriate revision, would a resubmitted manuscript be most suited for publication:

[a] in EMBO reports

6. Please add any further comments you consider relevant:

Although the authors demonstrate in octr-1 mutants the regulation of XBP-1 splicing and its effect on survival, the results must be demonstrated with more than one approximation. The techniques used to demonstrate these observations have limitations, as also the quantifications used. The authors have to address specific concerns here mentioned.

Referee #2:

XBP-1 is a transcription factor that plays a central role in the unfolded protein response (UPR). Abu genes are an interesting group of genes that are induced only in XBP-1 deficient C. elegans by ER stress (tunicamycin treatment), but not in normal worms. ABU-1 was shown to have a protective function against ER stress, but its molecular function and the upstream signal are relatively poorly understood.

In a previous study, authors demonstrated that the ablation of OCTR-1, a G protein coupled receptor in neurons induced Abu genes (through unknown mechanisms), which endowed the protection of the mutant animals from P. aeruginosa infection. In the current study, authors demonstrated that inhibition of XBP-1 suppresses the enhanced resistance to P. aeruginosa infection of OCTR-1 deficient animals. They further showed that P. aeruginosa infection activates XBP-1 (through IRE1) at the adult stage but not during development. Given that ABU genes are commonly induced in tunicamycin treated xbp-1 mutant worms and P. aeruginosa-infected OCTR-1 mutant worms, the relationship between XBP-1 and OCTR-1 is an interesting topic to explore. However, overall data presented in the current manuscript is only preliminary.

Specific points

1. To understand the role of OCTR-1 in the UPR, it will be important to investigate how OCTR-1 deficiency induces ABU genes. Are perk or atf6 activated in OCTR-1 mutant worms? ABU gene induction and the susceptibility to P. aeruginosa in compound mutants of octr-1/perk and octr-1/atf6 need to be investigated.

2. Does OCTR-1 deficiency cause basal ER stress, given that ABU genes are induced in OCTR-1 mutant worms? Are ABU genes also induced by tunicamycin treatment in OCTR-1 mutant worms?

3. It is confusing that XBP-1 and OCTR-1 have opposite roles in P. aeruginosa infection, while ABU genes are induced in both mutant strains.

4. In Figure 1, authors should show XBP-1 splicing in E. coli fed animals as a control.

5. In Figure 1B and 1E, splicing of XBP-1 in WT/PA14 is very modest compared with Tm treatment. Western blot of XBP-1s would be informative.

6. In figure 1B, XBP-1 splicing in Tm treated animals is minimal (and the negative control is missing) in L4 stage. Does IRE1 expression or activation (phosphorylation) vary during the development? Why is XBP1 splicing different between developing worms and adults?

7. Typos: page 5, line 1, XPB-1; page 4, last paragraph, IRE

Referee #3:

The UPR is important for effective immune response in C. elegans. In "Temporal and Organismal Regulation of XBP-1 Mediated Unfolded Protein Response," the authors show that the GPCR, OCTR-1, negatively regulates the UPR through the XBP-1-dependent pathway, but only during adulthood. In previous work the authors had shown that OCTR-1 suppresses the XBP-1 independent pathway. Overall, this is interesting work, which potentially provides insight into the observation that the L4 stage is generally more resistant to pathogen than the adult stage. However, some problems with the data presentation are noted that need to be corrected. Additionally, some suggestions for polishing the writing are given.

Data Issues

1. Figure 1A & 1D. These two panels of Figure 1 are comparing gene expression in the L4 (A) to young adult (D) in both the wt and octr-1 backgrounds. What is displeasing is that many more of the relevant genes are examined in D compared to A. The same set of genes that are thought to be relevant to the XBP-1-dependent part of the UPR should be examined under both conditions (L4, young adult) and the data shown in these panels.
2. Figure 1B & 1E. Missing a loading control. Use of an internal control, such as act-1 (which is used as a control in the qRT-PCR experiments), would work.
3. Figures 2 & 3. No information is provided on how many worms were scored to generate the data in 2B and 3B. It's not in the Figure Legends nor the Materials and Methods. Please add this information to the manuscript.

Writing Issues (please include page numbers on future manuscripts to increase ease of providing feedback)

1. Abstract: Third sentence a mess. Suggest breaking up the ideas to clarify. "There is also a canonical UPR pathway controlled by XBP-1. In this study we show that the XBP-1-regulated UPR pathway is controlled at the organismal level by OCTR-1. Importantly, OCTR-1's control of this signaling occurs only at the adult stage, not during development, and is independent of the presence or absence of infection."
2. Third paragraph of Introduction, first sentence. "IRE" is not defined.
3. Second-to-last sentence of introduction. Run-on sentence. Suggest breaking it up. Suggest deleting "early" from the sentence that follows (the changes are during development - not early development).
4. The first section of Results and Discussion needs an introductory sentence. I was really confused about why you were looking at L4 animals until I finally got to the part where you started looking at adult animals too and comparing the two stages. Suggest "To investigate whether XBP-1 is controlled by the nervous system differently depending on the stage, we looked at the activity of XBP-1, by various means, at the L4 stage compared to the young adult stage during infection. We first studied whether the transcriptional . . . "

1st Revision - authors' response 01 June 2012

Response to all reviewers

We would like to thank the reviewers for their thoughtful comments on our manuscript. We have responded to each of the reviewers' comments and suggestions by performing additional experiments and including changes to the paper. Four recent, very solid papers in the field dealing with XBP-1 use different approaches to address XBP-1 activation [PLoS Genet. 2011 Nov;7(11):e1002391; Nature. 2010 Feb 25;463(7284):1092-5; PNAS 2010 May 25;107(21):9730-5; PLoS Pathog. 2008 Oct;4(10):e1000176]: 1) *xbp-1* splicing, 2) qRT-PCR to measure the expression of XBP-1-dependent genes, and 3) images of an *hsp-4::gfp* reporter strain. In addition to using all these approaches, we have used a COPAS Biosort instrument to measure the fluorescence emitted by the *hsp-4::gfp* reporter strain. Thus, in an attempt to address the criticisms by the reviewers, we have gone beyond the current standards in our field. We are grateful for the critiques, which have made the paper much stronger, and hope the reviewers find our responses satisfactory.

Referee #1:

1. Do the contents of this manuscript report a single key finding? YES / NO
Yes, the manuscript "Temporal and organismal regulation of XBP-1-mediated unfolded protein response" by Jingru Sun and colleagues, determines the effects of mutant octr-1 in C. elegans on the regulation of UPR pathway that includes the transcription factor XBP-1. They describe this regulation at the temporal level because they show the splicing of XBP-1 only at adult stage not during development, and this correlates with the increased levels of target genes like hsp-4 and Y41C4A.11.

We thank the reviewer for an excellent summary of our study.

2. Is the main message supported by compelling experimental evidence? YES / NO
No, overall this is a well-done study that provides new and useful information about the regulation of splicing of XBP-1 on the development of C. elegans by octr-1; however, additional experiments and controls will be useful to explain the phenomena.

As explained below, we have included all the controls and performed all the additional experiments requested by the reviewer.

1.- The authors compared by qPCRs levels of targets genes that are dependent and independent of XBP-1 and its splicing, on mutants of octr-1 at basal levels and stimulated with P. aeruginosa, at two different stages of development. It is necessary to see if the genes showed in 1A and 1B changes and they have to include the splicing of XBP-1 at the same stages.

As requested, we have used qRT-PCRs to compare the levels of the same set of genes in both cases (New Figs 1A and 1D). In addition, the same conditions are used for the study of *xbp-1* splicing (New Figs 1B, C, E, and F).

2.- Images 1B and 1E are over-exposed to be quantified, and the quantification on 1C and 1F should shown as the ratio of spliced XBP1/ total XBP1, being total XBP1 the addition of spliced XBP1 plus unspliced XBP1. Also, they should include control samples: wt and octr-1 at basal levels.

We have performed the experiments requested by the reviewer. The experiments were replicated using WT and *octr-1* at basal levels (New Figs. 1 B and E). The exposure time was also reduced. Regarding the splice/unspliced ratio, we had performed the quantifications as in previous publication in our field [Henis-Korenblit et al. Proc Natl Acad Sci U S A. 2010 May 25;107(21):9730-5]. In deference to the reviewer's suggestion, we have performed the quantification as the ratio of spliced *xbp-1*/total *xbp-1* as requested (New Figs C and F).

3.-On figure 2 and 3, showing only one animal per stage of development is not enough to depict the phenomena, and it is necessary include what is the criteria of selection of ROI on Figure 2 and Figure 3.

As indicated in the new legend, animals that best represent the fluorescence level of the population were shown. We have also explained that the ROI corresponds to the entire animal. More importantly, to further address this comment, we have performed new experiments and quantified the fluorescence of hundreds of individual animals using a COPAS Biosort (New Supplementary Fig 2).

4.- Figure 2 and 3 can be evaluated the expression of GFP by Western Blot to validated the results obtained by fluorescence intensity.

We do not believe that the quantification of GFP by western blot would add much to the quantification of GFP fluorescence. However, we do appreciate the importance of quantifying a large number of animals. Thus, we have performed new experiments and quantified the fluorescence of hundreds of individual animals using a COPAS Biosort (New Sup. Fig 2).

3. Have similar findings been reported elsewhere (e.g. on a closely related protein; in another organism or context)? YES / NO

No.

4. Is the main finding of general interest to molecular biologists? YES / NO
Yes, it determined the regulation on the development process related to XBP-1. It has been described XBP-1 is necessary for the homeostasis, development of immune system and secretory organs.

We appreciate the interest of the reviewer in our study.

5. After appropriate revision, would a resubmitted manuscript be most suited for publication:
[a] in EMBO reports

6. Please add any further comments you consider relevant:
Although the authors demonstrate in octr-1 mutants the regulation of XBP-1 splicing and its effect on survival, the results must be demonstrated with more than one approximation. The techniques used to demonstrate these observations have limitations, as also the quantifications used. The authors have to address specific concerns here mentioned.

All the suggested experiments have been performed. As explained in the first paragraph of this document (response to all reviewers), we believe that in this study we have taken advantage of all the methodologies used by colleagues in the field. In addition, we have used additional approaches to fully address the criticisms of the reviewer.

Referee #2:

XBP-1 is a transcription factor that plays a central role in the unfolded protein response (UPR). Abu genes are an interesting group of genes that are induced only in XBP-1 deficient C. elegans by ER stress (tunicamycin treatment), but not in normal worms. ABU-1 was shown to have a protective function against ER stress, but its molecular function and the upstream signal are relatively poorly understood.

In a previous study, authors demonstrated that the ablation of OCTR-1, a G protein coupled receptor in neurons induced Abu genes (through unknown mechanisms), which endowed the protection of the mutant animals from P. aeruginosa infection. In the current study, authors demonstrated that inhibition of XBP-1 suppresses the enhanced resistance to P. aeruginosa infection of OCTR-1 deficient animals. They further showed that P. aeruginosa infection activates XBP-1 (through IRE1) at the adult stage but not during development. Given that ABU genes are commonly induced in tunicamycin treated xbp-1 mutant worms and P. aeruginosa-infected OCTR-1 mutant worms, the relationship between XBP-1 and OCTR-1 is an interesting topic to explore. However, overall data presented in the current manuscript is only preliminary.

We thank the reviewer for an excellent summary of our study.

Specific points
1. To understand the role of OCTR-1 in the UPR, it will be important to investigate how OCTR-1 deficiency induces ABU genes. Are perk or atf6 activated in OCTR-1 mutant worms? ABU gene induction and the susceptibility to P. aeruginosa in compound mutants of octr-1/perk and octr-1/atf6 need to be investigated.

The investigation of the regulation of *abu* genes is outside the scope of this study entitled "Temporal and organismal regulation of XBP-1-mediated unfolded protein response." *abu* genes are not part of the canonical XBP-1 pathway studied here. We have already studied the role of OCTR-1 in the control of *abu* genes in a previous work [Sun et al. Science. 2011 May 6;332(6030):729-32].

We thank the reviewer for his/her comments regarding *abu* genes. They made us realized that we had not clearly explained in the manuscript that *abu* (activated in blocked unfolded protein response genes) genes are activated by tunicamycin in animals lacking XBP-1. Page 4, last two lines and page 5, first two lines of the revised manuscript reads: "a family of genes classified as abu (activated in blocked unfolded protein response) because they are activated in xbp-1 mutant animals when ER

stress is induced by tunicamycin treatment [12, 13]. The abu genes encode UPR proteins that function in parallel with the canonical UPR pathway"

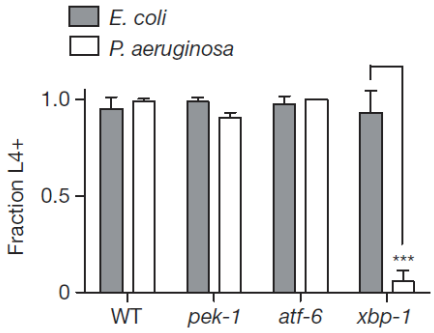

Fig. 1. Published results indicate that XBP-1 but not PEK-1 or ATF-6 is required for *C. elegans* survival on *P. aeruginosa*. Richardson et al. Nature. 2010 Feb 25;463(7284):1092-5

Since PERK and ATF6 do not play a role in response to *P. aeruginosa* infection [Richardson et al. Nature. 2010 Feb 25;463(7284):1092-5](Fig. 1 on the left), we do not believe they need to be further studied. In addition, the focus of this manuscript is on the XBP-1 branch of the UPR. As explained in the manuscript, while the cell autonomous mechanisms that sense ER stress and activate the XBP-1-mediated UPR pathway to prevent cellular damage and subsequent organismal failure have been elucidated, it is unknown whether XBP-1 is also controlled at the organismal level. This study represents the first demonstration that XBP-1 can be controlled at the organismal level by a GPCR that is neurally expressed.

2. Does OCTR-1 deficiency cause basal ER stress, given that ABU genes are induced in OCTR-1 mutant worms? Are ABU genes also induced by tunicamycin treatment in OCTR-1 mutant worms?

abu genes actually alleviate ER stress [Urano et al. J Cell Biol. 2002 Aug 19;158(4):639-46]. Therefore, there is no reason to believe that their upregulation in *octr-1* mutant animals would cause ER stress. As explained in the response to the previous comment, we have better described the *abu* gene family in the manuscript.

This study demonstrates that OCTR-1 controls XBP-1; it does not deal with the study of the control of ABU genes, which has been studied before by our laboratory [Sun et al. Science. 2011 May 6;332(6030):729-32].

3. It is confusing that XBP-1 and OCTR-1 have opposite roles in P. aeruginosa infection, while ABU genes are induced in both mutant strains.

We have better described the *abu* gene family in the manuscript (page 4, last two lines and page 5, first two lines)(please, also see response to comment #1). This should alleviate any confusion. OCTR-1 suppresses XBP-1 (canonical UPR) and *abu* genes (non-canonical UPR). *abu* genes are not induced in *xbp-1* mutants animals. *abu* genes are activated in *xbp-1* mutant animals only when ER stress is induced by tunicamycin treatment. Thus, it is expected XBP-1 and OCTR-1 to have opposite roles in defense against *P. aeruginosa* infection.

4. In Figure 1, authors should show XBP-1 splicing in E. coli fed animals as a control.

We have performed the requested experiments (New Figs. 1 B, C, E, and F).

5. In Figure 1B and 1E, splicing of XBP-1 in WT/PA14 is very modest compared with Tm treatment. Western blot of XBP-1s would be informative.

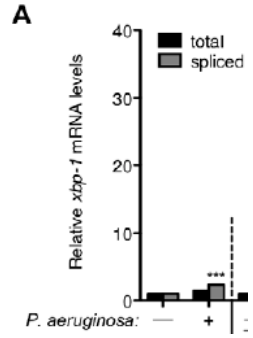

Fig. 2. A small induction of XBP-1 splicing by *P. aeruginosa* infection is shown. Data from Richardson et al. PLoS Genet. 2011 Nov;7(11):e1002391.

Unfortunately, there is not an antibody capable of recognizing *C. elegans* XBP-1. We and others have not detected any strong induction of XBP-1 by *P. aeruginosa* infection (Fig 2 on the left, Fig. 1 in our manuscript). In this study, we demonstrate that in the absence of OCTR-1, *xbp-1* splicing is induced in adult animals (Fig. 1E and F). When XBP-1 is not inhibited by OCTR-1, *P. aeruginosa* induces *xbp-1* splicing more strongly (Fig. 1F, octr-1/op50 vs. octr-1/pa14).

6. In figure 1B, XBP-1 splicing in Tm treated animals is minimal (and the negative control is missing) in L4 stage. Does IRE1 expression or activation (phosphorylation) vary during the development? Why is XBP1 splicing different between developing worms and adults?

We have repeated the experiments using all the controls in both cases (i.e., L4 and adult animals). We have studied XBP-1 activation by measuring its splicing, using qRT-PCR to quantify the gene expression levels of XBP-1-dependend genes, and by using a reporter line to study XBP-1 activation in vivo. We do not believe the study of the status of IRE1 is necessary in the context of this manuscript. IRE1 expression or its activation by phosphorylation has not been studied in other current papers that deal with XBP-1 in our field [PLoS Genet. 2011 Nov;7(11):e1002391; Nature. 2010 Feb 25;463(7284):1092-5; PNAS 2010 May 25;107(21):9730-5; PLoS Pathog. 2008 Oct;4(10):e1000176]. We have used the standards in the field to address activation of XBP-1.

We do not believe that XBP-1 splicing in developing animals is different than that in adults. The scales of Figures 1C and 1F are different, but the levels of splicing are not statistically different: L4 WT/OP50=3.66$\pm$0.49 vs. adults WT/OP50=4.64$\pm$0.08 (P value=0.122).

7. Typos: page 5, line 1, XPB-1; page 4, last paragraph, IRE

The typos have been corrected.

Referee #3:

The UPR is important for effective immune response in C. elegans. In "Temporal and Organismal Regulation of XBP-1 Mediated Unfolded Protein Response," the authors show that the GPCR, OCTR-1, negatively regulates the UPR through the XBP-1-dependent pathway, but only during adulthood. In previous work the authors had shown that OCTR-1 suppresses the XBP-1 independent pathway. Overall, this is interesting work, which potentially provides insight into the observation that the L4 stage is generally more resistant to pathogen than the adult stage. However, some problems with the data presentation are noted that need to be corrected. Additionally, some suggestions for polishing the writing are given.

We thank the reviewer for his/her interest in our study and the suggestions on how to improve the manuscript.

1. Figure 1A & 1D. These two panels of Figure 1 are comparing gene expression in the L4 (A) to young adult (D) in both the wt and octr-1 backgrounds. What is displeasing is that many more of the relevant genes are examined in D compared to A. The same set of genes that are thought to be relevant to the XBP-1-dependent part of the UPR should be examined under both conditions (L4, young adult) and the data shown in these panels.

We have repeated the experiments to be able to compare the levels of the same set of genes under both conditions as requested by the reviewer (New Figs 1A and 1D). In addition, the splicing experiments were also repeated so that the same conditions are used for L4 and adult animals (New Figs 1B, C, E, and F).

2. Figure 1B & 1E. Missing a loading control. Use of an internal control, such as act-1 (which is used as a control in the qRT-PCR experiments), would work.

As explained above, we repeated the experiments showed in Figs 1B and E adding a number of different conditions that are now similar in both cases (i.e., L4 and adults). We have also added the requested actin control.

3. Figures 2 & 3. No information is provided on how many worms were scored to generate the data in 2B and 3B. It's not in the Figure Legends nor the Materials and Methods. Please add this information to the manuscript.

10-20 animals were scored in Figs. 2 and 3. This information is in the revised legends. In addition, we have performed new experiments and quantified the fluorescence of hundreds of individual animals using a COPAS Biosort (New Supplementary Fig 2).

Writing Issues (please include page numbers on future manuscripts to increase ease of providing feedback)
1. Abstract: Third sentence a mess. Suggest breaking up the ideas to clarify. "There is also a canonical UPR pathway controlled by XBP-1. In this study we show that the XBP-1-regulated UPR pathway is controlled at the organismal level by OCTR-1. Importantly, OCTR-1's control of this signaling occurs only at the adult stage, not during development, and is independent of the presence or absence of infection."

We thank the reviewer for the suggestion. We have broken up the ideas in the abstract.

2. Third paragraph of Introduction, first sentence. "IRE" is not defined.

IRE has been defined.

3. Second-to-last sentence of introduction. Run-on sentence. Suggest breaking it up. Suggest deleting "early" from the sentence that follows (the changes are during development - not early development).

“early” has been deleted.

4. The first section of Results and Discussion needs an introductory sentence. I was really confused about why you were looking at L4 animals until I finally got to the part where you started looking at adult animals too and comparing the two stages. Suggest "To investigate whether XBP-1 is controlled by the nervous system differently depending on the stage, we looked at the activity of XBP-1, by various means, at the L4 stage compared to the young adult stage during infection. We first studied whether the transcriptional . . . "

We have modified the previous introductory sentence using the reviewer’s suggestion (page 4, Results and Discussion, first sentence). We really appreciate the effort of the reviewer to help us improve the manuscript.

2nd Editorial Decision 15 June 2012

Thank you for your patience while your revised manuscript has been under peer-review at EMBO reports. As you will see from the reports below, the referees are now all positive about its publication here, although referee 1 has three minor additional comments. I am therefore writing with an 'accept in principle' decision, which means that I will be happy to accept your manuscript for publication once referee 1's points have been addressed, including adding a brief explanation to the results described in figure 1, merging supplementary figure 3 into figure 2, and indicating the statistical test(s) used in the legends to figures 2B, 3B, 4 and SF2B.

Once all remaining corrections have been attended to, you will 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

REFEREE REPORTS:

Referee #1:

This paper demonstrates that XBP1 is regulated by OCTR1 in adult stage, but not during development, and this event correlates with the increase of target genes including hsp-4 and Y41C4A.11. The authors observed that OCTR-1 mutants animals has a enhanced resistance to P. Aeuriginosa infection and this is reverted with the inhibition of XBP1 expression (mutants and RNAi to XBP1). Together, the results of this paper establish that OCRT1 is a new regulator of XBP1 in nervous system in C. Elegans.
The authors have clearly improved the text in general, both the way in which results are presented as in the interpretation of them.
Only a few details could be addressed:

1.- Fig1: Include an explanation of why the comparisons were made only between L4 and the adult state. Did authors observe the same in the other stages of development?

2.- I suggest that FigS2 is included in Fig3. The graphs on FigS2 clearly show the differences on GFP expression on adult stage, they are very nice and support the work.

3.- The statistical test used should be mentioned figure legends.

Referee #2:

Authors convincingly demonstrated that OCTR1 deficiency in neuron activated noncanonical (abu) and canonical (xbp-1) UPR at the organism level, which conferred the resistance of OCTR1 deficient worm to bacterial infection.

It is intriguing how OCTR1 in neuron regulates UPR in other cells, and further study should identify the underlying mechanism.

Referee #3:

The authors are to be commended for very nicely addressing the reviewers' concerns. I have no further comments on this manuscript.

2nd Revision - authors' response 19 June 2012

Referee #1:
This paper demonstrates that XBP1 is regulated by OCTR1 in adult stage, but not during development, and this event correlates with the increase of target genes including hsp-4 and

Y41C4A.11. The authors observed that OCTR-1 mutants animals has a enhanced resistance to P. Aeuriginosa infection and this is reverted with the inhibition of XBP1 expression (mutants and RNAi to XBP1). Together, the results of this paper establish that OCRT1 is a new regulator of XBP1 in nervous system in C. Elegans.
The authors have clearly improved the text in general, both the way in which results are presented as in the interpretation of them.
Only a few details could be addressed:

1.- Fig1: Include an explanation of why the comparisons were made only between L4 and the adult state. Did authors observe the same in the other stages of development?

We decided to study L4 animals because they are at the last larval stage before becoming adults. We mention this in the revised manuscript.

Our results show that OCTR-1 does not control XBP-1 at any of the other larval stages (Figure 3 and Figure S3).

2.- I suggest that FigS2 is included in Fig3. The graphs on FigS2 clearly show the differences on GFP expression on adult stage, they are very nice and support the work.

We agree with the reviewer that the sorter data clearly show the differences in GFP expression. The sorter data support the results shown in both Fig 2 and Fig 3 (not only Fig 3). Thus, we decided to merge part of the sorter results with Fig. 2 (New Fig 2C). We have chosen to leave the rest of the sorter panels in the supplementary figure, because we believe that they do not add much to the images and their quantification shown in Fig 3.

3.- The statistical test used should be mentioned figure legends.

We believe that this information makes the legends too large and that it is a distraction. The statistical tests are mentioned in materials and methods in a section entitled “Statistical analysis”.

3rd Editorial Decision 19 June 2012

I am very pleased to accept your manuscript for publication in the next available issue of EMBO reports.

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

