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A yeast BH3-only protein mediates the mitochondrial pathway of apoptosis

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(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 12 May 2011

Thank you for transferring your manuscript for consideration by The EMBO Journal. As discussed, we have sent your manuscript to two arbitrating referees, who both saw the original referee comments from the previous journal and your detailed rebuttal. You will be pleased to learn that the two arbitrating referees both think that the points raised by the original referees have been addressed well, that there are no major concerns about the dataset that have not been brought up so far and that the mechanistic depth of the study is sufficient to justify publication in The EMBO Journal.

A number of points will require further attention:

Both arbitrating referees put forward a number of minor points that should be addressed:

Arbitrating referee 1:

Minor points
1. page 6: the PTP is not composed by ANT and VDAC, as the authors know very well. This was an operational model that has been surpassed when extensive genetic analysis ruled out that these molecules play any physical role in the formation of this channel. Cyclophilin D is conversely an important regulator of the PTP. Please rephrase this sentence to take the prevalent view into account and add all the appropriate references on ANT, VDAC and Ppif knockout mice and PTP.
2. page 6: it is not correct to conclude that PTP is not involved just because CsA fails to inhibit swelling. There are many examples of PTP mediated swellings that are insensitive to CsA, which is just a desensitizer of the channel. Please be more cautious when describing these findings: for example, it is correct to say that the permeabilization is CsA insensitive, not to say that it is PTP independent.
3. page 9: table S2, please remove the indication that the proteins are "component of the PTP" (see

above)

Arbitrating referee 2:

I would encourage the authors to minimize the use of the terms "ROS" and "oxidative stress" when discussing experiments using dyes such as DHE. Although DHE-> eth conversion may in fact be associated with ROS production, it is better to clarify that what is being measured is the oxidation of DHE and point out that superoxide and other ROS are known to promote this oxidation

Furthermore, there are a number of editorial issues that need to be dealt with. First, please check carefully at this stage that the manuscript is in EMBO J. format, and please add an author contribution section. Second, please include the statistical details for figure 3E, 3F, 3G, 3H, 3J as well as for supplementary figure S3A into the figure legends.

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

The full comments we received from the arbitrating referees were informal in nature.

1st Revision - authors' response 17 May 2011

Arbitrating referee 1:

Minor points

1. page 6: the PTP is not composed by ANT and VDAC, as the authors know very well. This was an operational model that has been surpassed when extensive genetic analysis ruled out that these molecules play any physical role in the formation of this channel. Cyclophilin D is conversely an important regulator of the PTP. Please rephrase this sentence to take the prevalent view into account and add all the appropriate references on ANT, VDAC and Ppif knockout mice and PTP.

> We thank the referee for this constructive comment. We changed the text and the references on page 6 as follows:
>
> "One controversial question is whether the permeability transition pore (PTP), a complex of proteins that consists of cyclophilin D as essential subunit, the adenine nucleotide translocase (ANT) as regulatory component and additional factors (Nakagawa et al., 2005;Baines et al., 2005;Schinzel et al., 2005;Baines, 2009), is required for BAX-induced apoptosis (Marzo et al., 1998;Shimizu et al., 1999;Kumarswamy and Chandna, 2009)."

2. page 6: it is not correct to conclude that PTP is not involved just because CsA fails to inhibit swelling. There are many examples of PTP mediated swellings that are insensitive to CsA, which is just a desensitizer of the channel. Please be more cautious when describing these findings: for example, it is correct to say that the permeabilization is CsA insensitive, not to say that it is PTP independent.

We agree and therefore substituted the phrase "PTP-independent" by "CsA-insensitive" in the results section as well as in the discussion. We now write on page 6:

"While pre-incubation of mitochondria with the PTP inhibitor cyclosporine A (CsA) severely reduced mitochondrial swelling promoted by Ca^{2+} (Fig. 3G, H), BH3 domain-induced swelling was unaffected (Fig. 3E, F and H), indicating that the yeast BH3 domain can permeabilize murine mitochondria through CsA-insensitive mechanism."

And on page 12 in the discussion:

"However, Ybh3p continued to execute death even in the absence of these proteins, and its BH3 domain induced swelling of isolated mouse mitochondria in a CsA-insensitive manner."

3. page 9: table S2, please remove the indication that the proteins are "component of the PTP" (see above)

We changed this according to the referee's suggestions. The respective paragraph on page 9 now reads as follows:

"The majority of isolated proteins were mitochondrial, including an adenine nucleotide translocase isoform as putative regulator of the PTP (Table S1). We determined the capacity of Ybh3p to induce ROS production and lethality in deletion mutants of all proteins that co-purified with FLAG-tagged Ybh3p."

Arbitrating referee 2:

I would encourage the authors to minimize the use of the terms "ROS" and "oxidative stress" when discussing experiments using dyes such as DHE. Although DHE-> eth conversion may in fact be associated with ROS production, it is better to clarify that what is being measured is the oxidation of DHE and point out that superoxide and other ROS are known to promote this oxidation

To clarify this point, we now mention the mean of quantification of ROS accumulation several times within the manuscript. We introduced on

Page 4
"…was markedly less effective in inducing ROS accumulation (indicated by DHE oxidation) and loss of clonogenic survival than full-length Ybh3p…"

Page 5
"Consistently, death and ROS-accumulation (evident by oxidation of DHE) induced by Ybh3p…"

Page 8
"Upon treatment with H_2O_2 or acetic acid, Dybh3 cells displayed reduced death, DHE-detectable ROS accumulation and apoptotic phosphatidylserine externalization…"

Page 8
"…deletion of YCA1 caused a strong decrease in ROS production (as indicated by DHE oxidation)."

Furthermore, there are a number of editorial issues that need to be dealt with. First, please check carefully at this stage that the manuscript is in EMBO J. format, and please add an author contribution section. Second, please include the statistical details for figure 3E, 3F, 3G, 3H, 3J as well as for supplementary figure S3A into the figure legends.

We changed the manuscript format according to the EMBO Journal guidelines and added an author contribution section, conflict of interest statement, keywords and running title.

The missing information in the figure legends for figure 3E-H, 3J and supplementary figure S3A has been included.

2nd Editorial Decision 20 May 2011

Thank you for sending us your revised manuscript. I have now had a chance to look at the revisions you made, and you will be pleased to learn that you have addressed all points in a satisfactory manner. The paper will now be publishable in The EMBO Journal and you will receive a formal acceptance letter shortly.

Thank you very much again for considering our journal for publication of your work.

Yours sincerely,

Editor
The EMBO Journal

