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Axonal Transcription Factors Signal Retrogradely In Lesioned Peripheral Nerve

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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 09 December 2011

Thank you for submitting your manuscript to the EMBO Journal. Your study has now been seen two referees whose comments are provided below. As you can see both referees find the analysis interesting, insightful and very well done. They both support publication of the study here as is and no further experiments are needed. Referee #2 has a few suggested text changes, but that is pretty much it. Regarding referee #2's suggestion to remove some of the data, I would prefer to keep the data in, but we can discuss this further. Given the referees' comments, I would like to invite you to submit a revised manuscript that addresses the minor issues raised.

A few editorial comments: please make sure that the array data is deposited in a database (see guide to authors) and that the accession codes are provided in the manuscript. Also, we now encourage the publication of source data, particularly for electrophoretic gels and blots, with the aim of making primary data more accessible and transparent to the reader. Would you be willing to provide a single PDF file comprising the original, uncropped and unprocessed scans of all or key gels used in the figures? These should be labeled with the appropriate figure/panel number, and should have molecular weight markers; further annotation could be useful but is not essential. This PDF will be published online with the article as a supplementary "Source Data" file. If you have any questions regarding this just contact me.

Thank you for submitting your interesting study to the EMBO Journal!

Editor
The EMBO Journal

REFEREE REPORTS

Referee #1

This manuscript by Keren Ben-Yaakov et al. explores the role of STAT3 retrograde axonal transport in modulating survival of peripheral sensory neurons after injury. This is an exceptionally well-written manuscript that provides very interesting and important information regarding the role of transcription factors in communicating information about injury between the axon and the nucleus. The remarkable quality and breadth of the experiments fully support the conclusions. This manuscript is of high quality and high impact and is suited for publication in the EMBO J.

Referee #2

This paper from Ben-Yakoov investigates the role of specific transcription factors in the response to nerve injury and provides overwhelming evidence of the involvement of activated STAT3 in this process.
By a comprehensive array of in vitro and in vivo approaches, the authors defined the mechanism of local translation of STAT3, its recruitment to the main retrograde motor complex and the downstream effects on gene regulation.
The experiments are logical, well performed and the results in general very clear. As a result, the conclusions of this paper are well supported by the experimental evidence, which are presented in a concise, yet very effective writing style.
As such, it is very difficult to find areas of improvements in this work. However, if a minor criticism may be made, this is that the richness of data, especially supplemental material, may distract the reader from the main story line.
However, this is a refreshing piece of news in times in which authors tend to present small accounts of their discoveries. Therefore, the authors should ultimately decide if this is the final format in which they wish their work to be published.
In addition to possible trimming of the data (for example Figure 1A-C, supplemental Figure 1-3, Figure 4B top part, Figure 5B to cite a few), the authors might consider adding some clarification in the text regarding the points below:
1. What do the multiple bars labelled with STAT and SMAD refer to in Figure 1D?
2. Page 5. Have the SMAD been validated as well? The statement at page 10 that STAT3 is the most robust candidate suggests that a comparative analysis has already been done. Please clarify.
3. In contrast to the high quality of the experimental data, the bands shown in Figure 3H look rather artificial and should be replaced with a higher quality scan.
4. Which isoforms of STAT3 (alpha or beta) is phosphorylated in vitro and in vivo? Only a single band is visible in blots detected with a phospho-specific antibody (e.g. Figure 3 and 4A).
5. What is the physiological significance of the interaction of unphosphorylated STAT3 with cytoplasmic dynein and its axonal retrograde transport?
6. The authors stated at page 9 that the classical NLS may compete with the binding of several other factors to the complex. Is this just a possibility or could any support for this hypothesis be provided in the discussion?
7. The model described in Figure 7 is not sufficiently highlighted in the text.
8. Could STAT3 or STATs be mentioned in the title?

1st Revision - authors' response 15 December 2011

Referee #2

However, if a minor criticism may be made, this is that the richness of data, especially supplemental material, may distract the reader from the main story line. However, this is a refreshing piece of news in times in which authors tend to present small accounts of their discoveries. Therefore, the authors should ultimately decide if this is the final format in which they wish their work to be published. In addition to possible trimming of the data (for example Figure 1A-C, supplemental Figure 1-3, Figure 4B top part, Figure 5B to cite a few…

Although we appreciate the reviewer's kind suggestion, we prefer to provide the data as a full package in this case. As referee #1 kindly noted, "*…the … breadth of the experiments fully support the conclusions…*", hence we would like the reader to receive the full breadth of the data..

1. What do the multiple bars labelled with STAT and SMAD refer to in Figure 1D?

Each bar represents a different TFBS probe, which the commercial company that produces the array claims represents an individual isoform in that transcription factor family (e.g. STAT1, STAT3, STAT4, STAT5). However due to the very high similarity between TFBS within the same factor family there is a high likelihood of cross-binding within the same family of probes – for example our biochemical validation data strongly suggests that any "STAT4" or "STAT5" signals on these arrays in our experiments were actually due to binding of activated STAT3. Hence we prefer not to specify individual TFBS designations in these cases. A clarification has been added to the figure legend.

2. Page 5. Have the SMAD been validated as well? The statement at page 10 that STAT3 is the most robust candidate suggests that a comparative analysis has already been done. Please clarify.

We have not yet done any validation of SMADs, and the statement on page 10 was meant to refer only to the STAT family. The sentence has been reworded for clarity.

3. In contrast to the high quality of the experimental data, the bands shown in Figure 3H look rather artificial and should be replaced with a higher quality scan.

We apologize for the poor quality of this panel in the original figure. It has now been replaced with a higher quality scan.

4. Which isoforms of STAT3 (alpha or beta) is phosphorylated in vitro and in vivo? Only a single band is visible in blots detected with a phospho-specific antibody (e.g. Figure 3 and 4A).

As noted in the text, the main band detected in most cases is STAT3alpha, since the beta form is expressed at lower levels. In some cases where only a single band is seen (corresponding to the alpha isoform), overexposure of the blots can reveal a minor occurrence of the beta isoform as well.

5. What is the physiological significance of the interaction of unphosphorylated STAT3 with cytoplasmic dynein and its axonal retrograde transport?

We did not monitor axonal retrograde transport of unphosphorylated STAT3, although it is reasonable to assume (as the reviewer apparently does) that if one sees it in a dynein pull-down it may be retrogradely transported. We do not know at this stage if there is any physiological significance to this observation.

6. The authors stated at page 9 that the classical NLS may compete with the binding of several other factors to the complex. Is this just a possibility or could any support for this hypothesis be provided in the discussion?

As noted in the discussion (page 10, bottom), "*the fact that a classical NLS peptide did not inhibit retrograde transport of STAT3 indicates that both types of cargos do not compete…*". Also at the end of the discussion (page 12) we note – "*The involvement of additional TFs in the retrograde injury response is also supported by the more potent effects on neurite outgrowth we observed upon injection of the NLS peptide (Supplementary Figure S8). These might include Max, PPARs or Smads, as well as other yet unidentified cargos. Additional work will be required to delineate the full complement of retrogradely transported TFs in axons, and to determine the range of physiological phenomena impacted by this direct route from axon to nucleus*". We would prefer not to add any further speculations, since the nature of the cargo(s) perturbed by classical NLS peptides is still under investigation.

7. The model described in Figure 7 is not sufficiently highlighted in the text.

We have added another reference to the model at an appropriate point in the discussion (Page 12, line 3).

8. Could STAT3 or STATs be mentioned in the title?

We would prefer to leave the title as is, to emphasize the likely generality of this mechanism. STAT3 is specifically mentioned in the abstract, and STAT is in keywords.

