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PTK7/Otk interacts with Wnts and inhibits canonical Wnt signaling

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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.)

Transfer Note 29 April 2011

PLEASE NOTE that this manuscript was transferred from a different journal and the two referees assessing suitability for The EMBO Journal had access to both the original anonymous comments as well as the point by point response from the authors.

Editorial Staff
The EMBO Journal

1st Editorial Decision 23 May 2011

Dear Dr. Tolwinski,

Thank you very much for transferring your research paper on PTK7/Otk in Wnt-signaling for consideration to The EMBO Journal editorial office. Having received consistent comments from two expert scientists, I am able to make a decision on your study to facilitate efficient proceedings. As you will see from the reports, both scientists appreciate the importance of your study. Although the molecular mechanisms seem not yet fully resolved, the interesting novel role of PTK7 in pathway selection appears of sufficient general interest to justify publication at this stage. I would still be grateful if you thoroughly discuss item 1 of the first referee and clarify/adjust presentation of item 2 that would make the paper also better accessible for a non-fly audience. Though not a condition for final acceptance, the inclusion of possibly existing more recent results that provide further insight into underlying molecular details would be highly welcome. With this, I am very much looking forward to receipt of a revised version for final assessment.

Please be reminded that it is EMBO Journal policy to allow a single round of revisions only and that the final decision still depends on content and strength of this version of your manuscript.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS:

Referee #1:

The authors show that the transmembrane receptor PTK7/Otk interacts with certain Wnt ligands and inhibits canonical Wnt signaling. Together with previous results that indicate an involvement of PTK7 in non-canonical Wnt signaling, this suggests a role for PTK7 as a determinant of pathway selection. The manuscript contains a number of interesting findings and interpretations, and it is generally well written. However, major problems remained unresolved.

1- It is implied that a complex is formed that includes Wnt, Fz and PTK7/Otk. However, the nature of this complex, if it exists at all, remains vague, mainly because direct interactions between components are not demonstrated. In particular, claiming that "PTK7/Otk binds Wnts ...." in the title and in other prominent places is highly misleading, given that interaction with Wnts requires the presence of Fz. This suggests actually an indirect binding to PTK7. Moreover, in a previous paper (Shnitsar and Borchers, 2008), it had been shown that co-precipitation of Fz and PTK7 required the recruitment of Dsh, whereas in the present manuscript, it is demonstrated that (1) the extracellular domain of PTK7 (incapable of Dsh recruitment) is precipitated by Wnts, but that (2) co-precipitation of these factors nevertheless requires the presence of Fz7. Is Fz and PTK7 interaction now independent of Dsh when Wnts are present? Things seem not to add up here.

2- The results concerning the denticle rows in Drosophila should be better explained. For example, it is claimed that ectopic rows are formed in response to Otk overexpression, but from Fig.1C, it seems that the spacing between rows, not their number, is increased. Providing quantitative data (row counts) may help. Likewise, co-expression of Otk and Wnt4 uniformly in embryos is claimed to expand the region of denticle producing cells, but from Fig.1D-G it appears as if the space between denticle bands were reduced, and not the width of bands increased (more bands are seen within the same length of embryo). This should be explained. Quantitative evaluations may be useful in all these experiments.

3- Inhibition of secondary axis induction/luciferase activation by PTK7 in Fig.5A-C appears to be rather mild. Were the doses of Wnt8 and PTK7 mRNA titrated to give optimal results? How can the weak inhibition be explained?

In summary, although potentially very interesting effects are described that shed new light on PTK7/Otk function in Wnt signaling, these effects are not sufficiently understood at a mechanistic level to warrant publication of the manuscript in its present form in the EMBO Journal.

Referee #2:

This manuscript presents data supporting a model in which the transmembrane receptor PTK7 (or Otk in Drosophila) functions in non-canonical Wnt signaling by turning off the canonical signaling branch. The inhibitory activity of PTK7/Otk on the canonical Wnt/Wg pathway has been well documented by the authors, including binding data on the ligand/receptor interactions. The experiments (overexpression and some loss of function) were done in both Drosophila and Xenopus suggesting the underlying mechanism is conserved.

While the mechanism is not (yet) precisely defined, in terms of its scope and impact the story is well suited to EMBO Journal; indeed I feel that the data are very interesting for the field. Moreover based on the comments of earlier reviewers and the responses of the authors (related to submission at another journal) it is my opinion that the manuscript has been adequately polished and is suitable for being published in EMBO Journal without further changes or reviewing.

1st Revision - Authors' Response 16 June 2011

Referee #1:

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1- It is implied that a complex is formed that includes Wnt, Fz and PTK7/Otk. However, the nature of this complex, if it exists at all, remains vague, mainly because direct interactions between components are not demonstrated. In particular, claiming that "PTK7/Otk binds Wnts ...." in the title and in other prominent places is highly misleading, given that interaction with Wnts requires the presence of Fz. This suggests actually an indirect binding to PTK7.

We agree with the reviewer that the use of the term "PTK7/Otk binds Wnt" may be misleading and replaced this now with "co-precipitates" or "interacts" throughout the manuscript.

Moreover, in a previous paper (Shnitsar and Borchers, 2008), it had been shown that co-precipitation of Fz and PTK7 required the recruitment of Dsh, whereas in the present manuscript, it is demonstrated that (1) the extracellular domain of PTK7 (incapable of Dsh recruitment) is precipitated by Wnts, but that (2) co-precipitation of these factors nevertheless requires the presence of Fz7. Is Fz and PTK7 interaction now independent of Dsh when Wnts are present? Things seem not to add up here.

Although the previous paper may suggest that the Fz/PTK7 interaction is more robust in the presence of Dsh in Xenopus lysates (Fig. 1), there is no evidence that this interaction requires Dsh. On the contrary, in human cell culture we find evidence that the Fz7/PTK7 interaction is independent of Dsh binding: like full length PTK7, the kinase deletion mutant of PTK7 (ΔkPTK7), which does not recruit Dsh, co-precipitate Fz7. This experiment has now been added in Fig. 4F and the text has been changed accordingly on page 9.

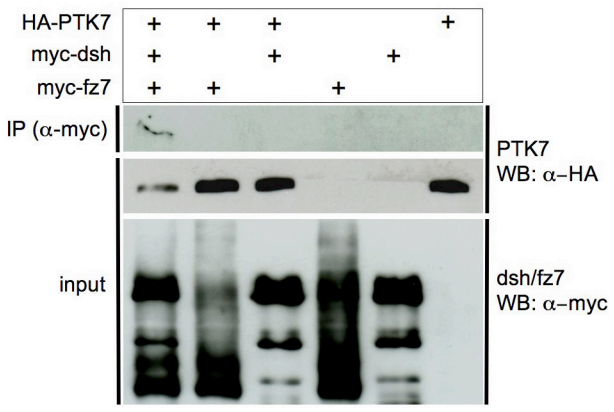

Figure 1: This is the data that was shown as supplementary Figure S2 in Shnitsar and Borchers, 2008. "**Fz7 and dsh are both required to co-precipitate PTK7.** Embryos were injected with 1 ng *PTK7-HA*, 0.2 ng *dsh-myc* and 0.2 ng *fz7-myc* RNA in combinations as indicated. Protein complexes were precipitated using anti-myc antibodies (IP). Immunoprecipitated HA-tagged PTK7 protein was detected by Western blotting using anti-HA antibody (upper panel). The middle panel shows the PTK7 input detected by Western blotting (WB) using anti-HA antibodies. The lower panel shows the dsh/fz inputs detected by anti-myc antibodies." Further, we stated in the manuscript text that HA-tagged PTK7 was co-precipitated with myc- tagged fz7 in 1 out of 3 experiments, but robust co-precipitation was only detected in combination with myc-tagged dsh and myc-tagged fz7.

2- The results concerning the denticle rows in Drosophila should be better explained. For example, it is claimed that ectopic rows are formed in response to Otk overexpression, but from Fig.1C, it seems that the spacing between rows, not their number, is increased. Providing quantitative data (row counts) may help. Likewise, co-expression of Otk and Wnt4 uniformly in embryos is claimed to expand the region of denticle producing cells, but from Fig.1D-G it appears as if the space between denticle bands were reduced, and not the width of bands increased (more bands are seen within the same length of embryo). This should be explained. Quantitative evaluations may be useful in all these experiments.

The cuticle assay for Wnt signaling is well established. Segment polarity genes were discovered using this assay as the last step in the anterior-posterior patterning cascade (Nusslein-Volhard and Wieschaus, 1980). Subsequently, these genes were cloned and assigned into the Wnt or Hedgehog signaling pathways and organized hierarchically through epistasis experiments. Along with the molecular marker Engrailed, this along with axis duplication in Xenopus embryos, led to our current understanding of the Wnt signaling pathway. The spacing of the denticles, the identity, size, cell-number are all interesting subjects for the specialist, but here we concentrate only on the simple signaling readout. To clarify the presentation of our data and to make these data more accessible for a non-fly audience we have added arrows to the figure, and changed the explanations to reflect the more obvious details such as more or fewer denticles. The explanation in the text has also been enhanced to make the assay clearer to the non-expert.

3- Inhibition of secondary axis induction/luciferase activation by PTK7 in Fig.5A-C appears to be rather mild. Were the doses of Wnt8 and PTK7 mRNA titrated to give optimal results? How can the weak inhibition be explained?

As with all RNA experiments, there is no single ideal mRNA concentration. However, we have tested a range of different RNA concentrations and settled on an experimental set up that reproducibly produced significant axis induction and minimized toxic effects. The inhibition of canonical Wnt signaling that we observe is significant and comparable to published data (Zhu et al., 2008 (Fig. 2B); Witte et al., 2010 (Fig. 4C)).

In summary, although potentially very interesting effects are described that shed new light on PTK7/Otk function in Wnt signaling, these effects are not sufficiently understood at a mechanistic level to warrant publication of the manuscript in its present form in the EMBO Journal.

We have corrected the errors that the referee points out. As PTK7 fails to act as a kinase, it is much more difficult to propose a definitive mechanism. We believe that since it behaves in much the same way as other proposed Wnt co-receptors (van Amerongen et al., 2008), although with opposing outcomes, our mechanism based on scaffolding is very likely to be correct.

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We thank the referee for the kind comments. This manuscript has indeed gone through several rounds of revision, and should now be ready for publication.

Reference:

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Zhu, W., Shiojima, I., Ito, Y., Li, Z., Ikeda, H., Yoshida, M., Naito, A. T., Nishi, J., Ueno, H., Umezawa, A. et al. (2008). IGFBP-4 is an inhibitor of canonical Wnt signalling required for cardiogenesis. *Nature* 454, 345-9.

