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Structure of the full human RXR/VDR nuclear receptor heterodimer complex with its DR3 target DNA

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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 31 October 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 rebuttals. You will be pleased to learn that the two arbitrating referees will both strongly support publication of the study here after some minor changes have been done (please see below). I would like to strongly encourage complying with arbitrating referee 2 and including the additional data of the 'old' figure 3 into the final version of the manuscript. In addition, I need to ask you to add an author contribution section as well as a conflict of interest statement into the main body of the text after the acknowledgement section and to provide the accession details of the EM Data Bank entry at this point.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Peer Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://www.nature.com/emboj/about/process.html

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

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

Referee #1

The study of Orlov provides the first Cro-EM structure of a nearly full-length nuclear receptor, and one of a handful of such structures with any technique.

Regarding the feasibility and technical aspects of the work:
This study is one of the smallest complexes studied with this technique. While rare, the limits on structure solution for this size appear to be technical (Glaeser and Hall, Biophys. J. Volume 100, Issue 10, 2011, Pages 2331-2337).

While not an expert on Cryo-EM, I was convinced by the data presented. They represent an ideal case for structure determination with small particle size:
1) The complex includes three components of different sizes and shapes with known structures and connectivity; 2) It contains DNA, which enhances contrast; 3) The clearly visible LBDs are off center from the response elements, aiding determination of polarity 4) The matching structure with an extended DNA sequence strongly validates the results.

Looking at the unbiased maps, the overall positioning of the LBDs versus DBD /DNA is obvious at first glance to someone familiar with the structures of the individual components. The VDR CTE and helix 1 are clearly visible in the figures, and different from RXR, allowing unambiguous assignment of polarity. The authors also describe other features that they used for discrimination. While these are not visible in the figures, they are again obvious in the structures used for docking, leaving no doubt about the correct docking.

Regarding the Biology: This structure is of critical importance for advancing our understanding of nuclear receptor structure and function, and more generally of understanding interdomain communication in allosteric signaling molecules.

This structure provides an important contrast to the one crystal structure of a full length NR, the RXR/PPARgamma/DNA complex. An important finding is that the DBD and LBDs are oriented differently with respect to each other, and the polarity on the DNA. This has important implications for understanding how the DNA can control binding of transcriptional coregulator proteins to the LBD, and how nuclear receptors might differ in this respect.

The have done a very good job addressing the comments from the many previous reviewers.

Some minor comments:
1. VDR AB domain is not clearly visualized, and this comment in the discussion should be removed.
2. Preferential binding of coactivators to the RXR partner-I believe this is not universally true. Some heterodimers preferentially utilize the RXR partner, including TR and LXR. It might be more accurate to say that heterodimers display different preferences for which partner contacts the coactivator, and this study shows how they also have different orientations of the coactivator binding sites relative to the DNA.
3. I stared at the supplemental figure 2 for a while but couldn't see stereo, which I usually can. I also could not see how this figure illustrates visualization of a helix in the maps. This might be improved by trying different visualizations of the ribbons, such as thinner, or partially transparent.

Referee #2

I support publication of the Klaholz paper, taking into account the earlier figures that were provided to me. However, I think that it is necessary to include the information in the old figure 3 as supplementary material, in order to justify the feasibility of particle identification and analysis. This will be a matter of considerable concern to readers who know about single particle EM.
I don't know what point is being illustrated by old Fig 3A, but B-E are certainly relevant as supporting information for this work. There is some repetition of panels in the main figures, but I would probably prefer to leave that so that at least some basic EM information is visible in the main

paper and the more technical comparisons are still made in the supplementary figure.
Overall I think the authors have done a good job and the work is suitable for EMBO J, even though the technical aspects might still be somewhat controversial.

1st Revision - authors' response 08 November 2011

Response to Referee 1 comments

We thank the referee for his/her enthusiastic feedback and thorough insights, both on the side of the biological impact being "of critical importance for advancing our understanding of nuclear receptor structure, function and allostery", as well as on the side of the technical advance and correctness of the cryo-EM work of "one of the smallest complexes studied" to date. The study indeed addresses the architecture of the entire RXR/VDR/DNA complex, the DNA polarity, inter-domain communication, and reveals an open architecture distinct from that of the rather compact crystal structure of PPAR/RXR (which in solution is also open as seen by SAXS analysis).

Feedback to the minor comments:

1) The visualisation of the VDR AB is actually rather good, e.g. in the stereo view of Fig. 1D and in Fig. 2A. We would suggest keeping the short discussion on the VDR AB domain in the text since it provides information on how to design future functional tests, particularly with respect to DNA binding.

2) With regards to the binding of co-activators, there is growing evidence that the RXR partner plays a key role there, for example SRC1 and Med1 bind to RAR or VDR in RXR heterodimers (Rochel et al., Nat. Struct. Mol. Biol. 2011), and we have more, yet unpublished evidence for that for other nuclear receptors also. Maybe there was some misunderstanding with the term "RXR-partner", we changed it to "partner of RXR" and included two references for that.

3) The small stereo representation in the Suppl. Fig. 2 is indeed in stereo, maybe it was less easy to see because the two small pictures were close to each other. We have separated them now more to facilitate stereo viewing. The arrows indicate the position of the RXR interface helix H10 as mentioned now in the main text and in the legend of Suppl. Fig. 2, which is resolved from the VDR helix H10 as visible in Suppl. Fig. 2 (middle panel).

Response to Referee 2 comments

We thank the referee for his/her very positive feedback. We are indeed happy to include additional figures in the supplementary material which illustrate the feasibility of particle identification and image processing. These figures include the cryo-EM visualization of particles under different defocus and acceleration voltage conditions, and the description of the independent *ab initio* structure determination of the nuclear receptor complex from 100kV and 200kV data. The additional figures are now discussed and/or referred to in the main text.

