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The RIG-I ATPase domain structure reveals insights into ATP dependent antiviral 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

08 Sep 2011

PLEASE NOTE that this manuscript was originally submitted to The EMBO Journal on 08. Aug. 2011 and transferred after peer-review to EMBO reports.

Editorial Staff
EMBO reports

Editorial Decision – The EMBO Journal

01 Sep 2011

Thank you for submitting your paper to the EMBO Journal. Your study has now been seen by three referees and their comments are provided below.

As you can see while there is an interest in the RIG-I helicase structure, there are also significant concerns with it that I am afraid preclude its publication here. The structural analysis is sound and there are not major technical concerns raised. However, the main concern is that the study does not go far enough to support the proposed model. In other words, we don't gain enough experimental

data to support the proposed conformational change that would have to occur to switch the helicase from an off state to an on state and there is limited mutational analysis to implicate movement of the "helical arm" in this process. Referee #3 also brings up a similar issue, although less strong. I have discussed the paper further with my colleagues and I am afraid that we are in agreement with referee #1 and that we unfortunately can't offer publication here.

Given the interest in this topic, I have taken the liberty of discussing your manuscript with an editor at EMBO Reports. EMBO reports is willing to commit to publishing your manuscript with minor changes. I sincerely hope that you will consider this option and transfer the paper to EMBO Reports. Time is very important here and we would need to move fast to facilitate a 2011 publication date.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS:

Referee #1:

RIG-I is an important helicase for the detection of viral RNAs, which are recognized via their 5' triphosphate group. RIG-I contains two CARD domains (involved in signaling), a helicase domain and an RD domain (involved in 5' triphosphate recognition). This work reports the high-resolution crystal structure of the helicase domain of RIG-I. It shows that the two RecA domains are in a non-productive conformation for RNA binding. The conformation observed in the crystal structure is consistent with solution scattering studies. The relative orientation of the two RecA domains is fixed by a rigid 'helical arm'. A comparison with known helicase structures suggests that a conformational change will have to occur to switch from this off state to an on state. How this is achieved is unclear, but the authors propose that it will likely require the movement of the 'helical arm'.

Overall, this is a solid structural work with biochemical data on the effect of mutations at the helicase active site. However, these biochemical experiments are largely redundant given the extensive body of data in the literature on the effect of mutating helicase active sites. I would have expected the authors to test the effect of mutating specific features of RIG-I. For example, mutations that interfere with the position of the 'helical arm' are expected to interfere with the off state of the molecule, according to their model. As it stands, I feel that the paper does not convey significant information on how this helicase works that would warrant publication in EMBO J.

Minor

- the model in Figure 6 - it is unclear how the two RecA domains are expected to change their relative orientation in the on and off state.

Referee #2:

In the present manuscript, Civril et al. report the crystal structure of a portion of mouse RIG-I in complex with AMPPNP. The investigated RIG-I fragment encompasses two RecA-like helicase domains connected by an unusual helical arm and a helical insertion domain in the second RecA-like domain. RIG-I is part of RIG-I-like receptor signaling systems, which are components of the innate immune system sensing pathogen-associated molecular patterns (PAMPs). RIG-I detects viral infections via pathogenic double-stranded RNAs bearing a 5'-triphosphate. The present work elucidates functional principles of RIG-I and provides a molecular framework on which to further explore how RIG-I activity is linked to downstream signaling. It should therefore be of interest a broad community interested in molecular mechanisms of RNA/RNP remodeling as well as host-pathogen interactions.

Civril et al. observe an unusual arrangement of the two RecA-like domains, which appears to be stabilized by the unusual helical connecting arm. The present arrangement seems to persist in solution as shown by SAXS analysis and corresponds to a "signal-off" state with functional helicase motifs misaligned. ATP binding alone is apparently insufficient to trigger a "signal-on" state since the current structure has been determined in the presence of AMPPNP. Motifs required for ATP hydrolysis are not properly aligned, explaining lack of ATPase activity in the signal-off state. Based on their structure, the authors can show how mutations in motif III that have previously been observed to render RIG-I constitutively active interfere with the positioning of the helical arm and thus may destabilize the observed auto-inhibited state.

Based on comparison with related superfamily 2 helicases, the authors conducted modeling studies, showing how a signal-on state can be adopted intramolecularly by repositioning of the second relative to the first RecA-like domain. The modeled on-state structure would require only small adjustments in the helical arm and would thus be in principle compatible with this molecular feature. The model predicts that highly conserved motifs (QQ and R) on the RIG-I-specific helical insertion domain would be repositioned to participate in RNA binding. The authors tested this prediction by mutating these motifs and observing strong effects on RNA-stimulated ATPase activity. Using directed mutagenesis, they also exclude the alternative possibility of a productive helicase conformation being produced in trans by RIG-I oligomerization. The functional roles of highly conserved residues in motifs Ic and IIa, predicted to participate in RNA binding, were also probed by mutagenesis. Mutation of these residues again abrogated RNA-stimulated ATPase activity. Finally, the authors suggest global models of how an additional RNA-binding domain (the regulatory/repressor domain; not contained in the structure) could recognize 5'-triphosphate and feed RNA to the helicase unit, and how two N-terminal caspase activation and recruitment domains (again not present in the structure) may exert their function.

The present work provides deep insights into the molecular mechanisms underlying signaling by RIG-I. A PAMP-induced conformational change is required for the protein to adopt an active helicase structure and the authors suggest and probe experimentally a plausible scenario for this conformational change. They discuss several possibilities how the helicase and RD domains could subsequently cooperate/interact to achieve downstream signaling.

The text is well written and nicely illustrated. The work appears to be technically sound and the results support the conclusions drawn.

Specific comments:

1. It appears from the description and the single entry in Table I that the structure is that of the SeMet protein. This is fine but should be briefly stated explicitly in the text.
2. Phasing statistics should be included in Table I.
3. Rwork/Rfree for the highest resolution shell should also be included in Table I.
4. The authors mention the comparatively "small interaction surfaces" between the domains of the present crystal structure (pg. 5, bottom). These interaction surfaces should be described in more detail - How large are they exactly? How do these areas compare to inter-domain interfaces in other helicases? What is their chemical nature (hydrophobic/hydrophilic)? Are they made up of conserved residues? The surfaces thought to change due to conformational rearrangements during signaling may be expected to be made up of conserved residues.
5. It is not clearly described with which protein the SAXS analysis was performed. Was the protein also treated with protease as for crystallization? If so, how was the proteolyzed protein purified for SAXS? If not, how would the SAXS data account for the additional domains present in the originally cloned and purified protein portion? Was AMPPNP present in the SAXS analysis?
6. It appears as if AMPPNP is bound in an unusual fashion with a register shift in the elements recognizing the phosphates. An interesting question is whether ATP binding would induce any conformational change (although it can apparently not induce the fully active helicase

conformation). Could the SAXS data or comparative proteolysis or CD analysis (with and without AMPPNP) be performed to detect conformational changes induced by nucleotide binding?

7. Pg. 9, par. 2, line 10: "electron" should be replaced by "electrophoretic".

8. Pg. 12, par. 1, line 4: A reference should be given for the statement "similar to what is seen with some DEAD box enzymes".

9. Perhaps the authors should discuss the possibility of a stable, locked signaling complex (Pg. 13, par. 1, lines 1-3) in light of other helicases providing a "locking" function in signaling complexes such as eIF4AIII in the exon junction complex (Science 313: 1968-72, 2006; Cell 126: 713-25, 2006)

Referee #3:

The DExH/D RNA helicase RIG-I is a key innate immune sensor of viral RNAs bearing 5'PPP moieties. Structurally, it possesses an SF2 helicase domain, two N-terminal CARD domains and a C-terminal repressor domain (RD). Previous structural studies have been limited to the RD and have revealed it as a primary binding site for 5'PPP-RNA. However, a full structure of the protein has not been published and it has therefore been unclear how the helicase domain contributes to RNA binding and how this integrates with ATP hydrolysis to promote downstream signaling. The manuscript by Civril et al. now presents the crystal structure of the RIG-I helicase domain in complex with a non-hydrolysable ATP analog. The data highlight the presence of an atypical elbow-containing arm, which the authors propose is important for maintaining RIG-I in an inactive conformation. The structure suggests a model involving a conformational switch in the helicase domain upon ligand binding, which is consistent with previous published data. ATP hydrolysis assays of wild type and mutant RIG-I support the model. Altogether, this work provides insight into the specifics of RIG-I catalytic activity.

There are some issues outstanding. The model for RNA binding to the helicase domain is not based on any data and should be strengthened by testing mutation of the putative key residues in assays for RNA binding (such as gel shift). Also, it is not clear whether the mutants tested in the ATPase assay really have no effect on RNA binding as it seems from the Methods that the RNA binding assays were carried out with intact protein, where the activity of the RD would dominate the reaction.

Minor comments

- 1) Abstract: the term AMP-PNP should be spelled out
- 2) Page 3: IFIT proteins are not considered PRRs.
- 3) Page 3: A number of references have been omitted in the introduction (e.g. Chiu et al. Cell (2009); Rehwinkel et al. Cell (2010))
- 4) Page 8: Further explanation on the SF2 DEAD box enzyme VASA should be provided.
- 5) The authors could speculate where the tandem RIG-I CARDs are located within the structure.
- 6) As many others, the authors refer to RIG-I stimulatory RNA as "5'ppp double-stranded RNA". The use of the "ds" designation implies intermolecular base pairing between 2 strands of RNAs. However, it is well established that single-stranded RNA also activates RIG-I as long as it provides intramolecular base pairing close to the 5'-end (as found in "pan-handle" structures formed by viral RNAs). For the sake of clarity, we urge the authors to use the term "base paired" RNA.

Initial Correspondence – EMBO reports

05 Sep 2011

Thank you for considering a transfer of your study to EMBO reports. I have been in touch with referee 3 of The EMBO Journal submission and it seems there is a bit of confusion. It seems the data presented in figures 3D and SF2B were indeed done in the context of the full-length protein. Please

make this clearer in the revised manuscript. The bottom line of this referee's concern is that, if the mutants used in the ATPase activity assay were in the context of the full-length protein, the RNA binding activity of RD would dominate and no definite conclusions can be drawn regarding RNA binding, this -and the benefits of using the ATPase binding assay in this case- should be more clearly discussed in the manuscript.

Please also address the other minor points regarding the text, but keeping in mind that the piece needs to be reformatted to EMBO reports style. The length of revised manuscripts must be a maximum of 30,000 characters (including spaces, figure legends and references), and thus you will need to shorten the main text. Shortening may be made easier by combining the Results and Discussion into a single section, which we require, and which will help eliminate the redundancy that is inevitable when discussing the same experiments twice. In addition, although basic Materials and Methods required for understanding the experiments performed must remain in the main text, additional detailed information may be included as Supplementary Material. In addition, we can publish only a maximum of 5 figures in the main text and you currently have 6. In this case, I would suggest to combine figures 5 and 6 into a single one.

Do not hesitate to get in touch if I can be of further help. As you know, this is a time-sensitive case and, thus, I look forward to receiving your final version by next Thursday.

Yours sincerely,

Editor
EMBO Reports

Revision - authors' response

08 Sep 2011

Reviewer #1

We thank the reviewer for evaluating our work. He pointed out a minor issue which we have explained below.

Minor

- the model in Figure 6 - it is unclear how the two RecA domains are expected to change their relative orientation in the on and off state.

It is our intention that Figure 6 provides a model for how RIG-I might be activated by RNA binding, rather than how ATP hydrolysis might occur. Because it is not clear from our structure exactly how the two RecA domains might come together to form a functional active site, we have avoided showing this in the model. However RecA helicases have been extensively reported on in the literature, and it is well known that the two domains must come together to allow ATP hydrolysis to occur. In our revised manuscript Figure 6 has been renumbered as Figure 5.

Referee #2:

We thank the referee for a very positive evaluation of our work. The reviewer raised some points and we have done our best to follow the recommendations.

1. It appears from the description and the single entry in Table I that the structure is that of the SeMet protein. This is fine but should be briefly stated explicitly in the text.

We have followed the suggestion and stated it in the main text.

2. Phasing statistics should be included in Table I.

We have now included it in Table I.

3. Rwork/Rfree for the highest resolution shell should also be included in Table I.

We have changed the Table I as suggested by the referee.

4. The authors mention the comparatively "small interaction surfaces" between the domains of the present crystal structure (pg. 5, bottom). These interaction surfaces should be described in more detail - How large are they exactly? How do these areas compare to inter-domain interfaces in other helicases? What is their chemical nature (hydrophobic/hydrophilic)? Are they made up of conserved residues? The surfaces thought to change due to conformational rearrangements during signaling may be expected to be made up of conserved residues.

We have described the interfaces more precisely. They are mainly hydrophobic in nature, and appear to be conserved amongst RIG-Is from different species. We feel that, due to the brevity required for resubmission to EMBO Reports, that a full analysis of the interfaces is not possible in the context of this manuscript.

5. It is not clearly described with which protein the SAXS analysis was performed. Was the protein also treated with protease as for crystallization? If so, how was the proteolyzed protein purified for SAXS? If not, how would the SAXS data account for the additional domains present in the originally cloned and purified protein portion? Was AMPPNP present in the SAXS analysis?

It is indicated in Materials and Methods that the human RIG-I SF2 domain is the construct previously used in Cui et. al. 2008. The manuscript has been updated to explicitly mention that the SAXS analysis was carried out in the absence of any nucleotide.

6. It appears as if AMPPNP is bound in an unusual fashion with a register shift in the elements recognizing the phosphates. An interesting question is whether ATP binding would induce any conformational change (although it can apparently not induce the fully active helicase conformation). Could the SAXS data or comparative proteolysis or CD analysis (with and without AMPPNP) be performed to detect conformational changes induced by nucleotide binding?

This is a very interesting issue. However, the well fitting comparison of crystal structure (with nucleotide) and SAXS data (without nucleotide) confirms that nucleotide binding does not cause conformational changes. We have now clarified this in the text.

7. Pg. 9, par. 2, line 10: "electron" should be replaced by "electrophoretic".

We have corrected this typographical error.

8. Pg. 12, par. 1, line 4: A reference should be given for the statement "similar to what is seen with some DEAD box enzymes".

This part of the text has been removed from the manuscript.

9. Perhaps the authors should discuss the possibility of a stable, locked signaling complex (Pg. 13, par. 1, lines 1-3) in light of other helicases providing a "locking" function in signaling complexes such as eIF4AIII in the exon junction complex (Science 313: 1968-72, 2006; Cell 126: 713-25, 2006)

While this is an interesting suggestion, we feel our reduced EMBO reports style of manuscript does not have sufficient room to discuss this possibility.

Referee #3:

We are thankful to the referee for positive evaluation of our data. The referee raised some issues which we have tried to answer.

There are some issues outstanding. The model for RNA binding to the helicase domain is not based on any data and should be strengthened by testing mutation of the putative key residues in assays for RNA binding (such as gel shift). Also, it is not clear whether the mutants tested in the ATPase assay really have no effect on RNA binding as it seems from the Methods that the RNA binding assays were carried out with intact protein, where the activity of the RD would dominate the reaction.

We have updated the manuscript to more clearly explain the use of the ATPase assay to study RNA binding. In particular a new paragraph has been included as follows:

“We used the dsRNA stimulated ATPase activity to indirectly probe the ATPase motifs of SF2 along with surface residues important for RNA interaction. Most of the dsRNA binding activity of RIG-I is contributed by RD which thus masks effects of mutations in SF2 in a direct RNA binding assay (Suppl. Fig. 2A). Unfortunately, without RD SF2 alone is mostly inactive because it needs RD to present RNA for binding. However, the ATPase activity of wtRIG-I is very sensitive to RNA induced conformations in SF2 and can therefore help map RNA binding motifs.”

Minor comments

1) Abstract: the term AMP-PNP should be spelled out

We have replaced the term “ANP-PNP” with “ nucleotide analogue” to meet the requirements for brevity of EMBO Reports.

2) Page 3: IFIT proteins are not considered PRRs.

As suggested we have removed the IFIT from the list of PRRs.

3) Page 3: A number of references have been omitted in the introduction (e.g. Chiu et al. Cell (2009); Rehwinkel et al. Cell (2010))

We are grateful to be reminded of these references. However as our manuscript has been greatly reduced in size as part of the requirements for EMBO Reports, we are no longer attempting to make a full review of the field in our introduction section.

4) Page 8: Further explanation on the SF2 DEAD box enzyme VASA should be provided.

We have used the structure of VASA because of two reasons: the analogy of RIG-I helicase domain to VASA and the availability of the structure bound to nucleic acids with properly aligned ATPase motifs. The latter was indicated in the text but not the analogy which now is highlighted. We believe that further information is unnecessary in the context of our shortened manuscript.

5) The authors could speculate where the tandem RIG-I CARDS are located within the structure.

We tried to avoid speculations about the RIG-I CARDS as our available data does not provide any clue about the possible location of CARDS and there is a considerably long linker connecting the CARDS to the N-terminus of SF2 domain which provides a wide range of locations unlike RD.

6) As many others, the authors refer to RIG-I stimulatory RNA as "5'ppp double-stranded RNA". The use of the "ds" designation implies intermolecular base pairing between 2 strands of RNAs. However, it is well established that single-stranded RNA also activates RIG-I as long as it provides intramolecular base pairing close to the 5'-end (as found in "pan-handle" structures formed by viral RNAs). For the sake of clarity, we urge the authors to use the term "base paired" RNA.

We have updated our introduction to reflect that RIG-I is activated by base paired RNA.

Thank you for your submission to EMBO reports. I think it adequately addresses the referee concerns and, thus, I am happy to accept your manuscript for publication in the next available issue of EMBO reports. Thank you for your contribution to our journal. As you know, publication is time-sensitive in this case, so please submit all necessary forms promptly so that your manuscript can be sent to our production office without any delay.

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,

Editor
EMBO Reports