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Structural basis for specific recognition of Lys 63-linked polyubiquitin chains by tandem UIMs of RAP80

Yusuke Sato, Azusa Yoshikawa, Hisatoshi Mimura, Masami Yamashita, Atsushi Yamagata

Corresponding author: Shuya Fukai, The University of Tokyo

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Transaction Report:

(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 11 May 2009

Thank you for submitting your paper to the EMBO Journal. Your study has now been seen by three referees and their comments to the authors are provided below. As you can see there is interest in the paper, however one issue of concern is also a recent paper published in Mol Cell by Sims and Cohen. The concern raised here is if we gain enough new insight over this work. The Sims and Cohen paper is also not cited although it was published before submission of your study and is very relevant for the present findings. While referee #3 is not persuaded that the novel insight provided is sufficient to consider publication in the EMBO Journal, both referee #1 and 2 are more supportive of the analysis. I also went back to referee #2 and asked this referee to specifically comment on the advance over the Mol Cell paper. Referee #2, in agreement with referee #1, finds that the present analysis is a big step forward. Given the comments provided by both referee #1 and 2, I am going to go with their overall recommendation and ask you to submit a suitably revised manuscript that addresses the minor concerns raised by referee #1 and 2. Please also make sure to refer to the Sims and Cohen paper (and other papers published recently as indicated in the reviewers' comments) and include the relevant PDB codes in the revised manuscript. Time is also really important here and I would appreciate if we could receive the revised version as soon as possible. If you have any further questions do not hesitate to contact me.

When you send us your revision, please include a cover letter with an itemised list of all changes made, or your rebuttal, in response to comments from review.

Thank you for the opportunity to consider your work for publication. I look forward to reading the revised manuscript.

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REFEREE REPORTS

Referee #1 (Remarks to the Author):

Sato et al. solve the structure of Rap80's tandem UIMs complexed with K63-linked diUb by x-ray crystallography. They find this region of Rap80 to form a contiguous alpha-helix and that the region between the two UIMs plays a critical role in Rap80's K63 linkage specificity. This paper is of very high interest, due to Rap80's role in DNA repair; however, a recent 2009 Mol Cell paper by Sims and Cohen steels its thunder a bit. In the Sims/Cohen paper, domain swapping experiments are done to demonstrate the importance of the inter-UIM region of Rap80 for K63 linkage specificity and CD experiments help generate a model with the inter-UIM region helical. This reviewer feels that the experimental structure presented here is a significant step forward beyond the model presented in the Mol Cell paper however and favors publication of this manuscript in EMBO J if the following issues are resolved.

1. The paper needs to be brought up-to-date, especially in the introduction. For example, heterotypic chains also exist and K11 chains are abundant and signal for degradation. See Xu et al. Cell 2009 and Meierhofer et al. J. of Proteome 2008. And, as mentioned above, the Sims and Cohen paper should be referenced.

2. The authors make a convincing argument for why Rap80's UIMs require K63 linkages. But, it is not clear why UIM1 binds the proximal subunit while UIM2 binds the distal subunit. Are any other species present? If not, is this singular binding mode an artifact of crystallization? Could the reverse orientation with UIM1 on distal and UIM2 on proximal exist in solution?

Referee #2 (Remarks to the Author):

This manuscript reports the structure of the tandem UIM modules of RAP80 in complex with K63-linked di-ubiquitin. RAP80 is specific for K63-linked diUb and doesn't bind either linear diUb or K48-linked di-Ub. The authors find that the tandem UIM domains (UIM1 and UIM2) forms a continuous a-helix with each UIM binding to the hydrophobic patch centred on Ile44 of each Ub monomer. Interestingly, the isopeptide Gly76-K63 linkage of diUb does not contact RAP80. Swapping the order of UIM1 and UIM2 has little affect on binding and specificity, however, altering the linkage length between UIM1 and UIM2 abolishes binding, indicating that the rigid linker functions as a molecular ruler and defines the relative orientation of the two UIM domains. Finally, the authors characterise the three tandem UIMs of Epsin which they show are specific for Lys63-linked Ub, and that the linkage length is important, together with the amino acid composition of the UIM1-UIM2 linker.

This is an extremely interesting and significant manuscript that is well written with clear and important results. The question of ubiquitin chain-binding specificity is an important area of research, and this work makes an important contribution to the field. It is highly worthy of publication in EMBO J and given the interest and competition in this field should be published rapidly.

Some minor points:

1. RAP80 is specific for K63-linked di-Ub and doesn't bind linear diUb, even though the linkage between the two Ub moieties are not involved in binding to RAP80. Komander et al (EMBO Reports) have recently shown that K63-linked and linear di-Ub have very similar structures. Can the authors speculate on the basis for the K63-mediated specificity?
2. Recently Rahighi et al (Cell) published the structure of the UBIN domain of NEMO in complex with linear di-Ub. The authors should discuss this work.
3. I'd recommend that Supp Fig S2 is included in the main manuscript.

Referee #3 (Remarks to the Author):

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This study reveals the binding pattern of K63-linked Ub2 to the two UIMs of RAP80 from Mus musculus by resolving the crystal structure of the UIM-Ub-complex. From their data the authors conclude which amino acids of RAP80 and K63-linked Ub2 are involved in the binding showing that both UIMs recognize ubiquitin at the same surface around the Ile 44-surrounding hydrophobic patch. The UIM1 binds to the proximal Ub-moiety while the UIM2 binds in a similar fashion to the distal Ub of K63-linked Ub2.
By exchanging the UIMs artificially in different combinations (UIM1-UIM1; UIM2-UIM2; UIM2-UIM1) they could show that the selectivity of the binding to K63-linked Ub2 does not depend on the order of the UIMs, since each of the constructs bound to K63-linked Ub2. Rather, recognition of K63-linked Ub2 relies on the linker between the two UIMs.
The experiments from the authors reveal structural and biochemical but no physiological functional data. Since the concept of the importance of the linker is already known there is no novel insight gained with this study. If to the crystal structure additional physiological functional experiments and other issues explained below can answer relevant questions the study would be interesting. As it is right now the only novelty is the structure derived from crystallisation of RAP80-UIMs in complex with K63-Ub2. It is also unprofessional that the authors do not mention already published manuscript with the direct relevance to this finding.

Conceptual issues and questions:
1.) The dependency on the linker for binding specifically to K63-linked Ub2 is one of the main conclusions derived from the authors structural and biochemical analysis. However this concept has already been evaluated and published by Joshua Sims and Robert Cohen in "Molecular Cell" in March 2009. Sims and Cohen modelled a structure for the UIMs and the linker, which looks very similar to the crystal, but their work is not even mentioned once. A comparison of the author's data with the already published one is therefore mandatory.
2.) The specificity for K63-linked Ub2 should be elaborated in more detail. Komander et al. (EMBO rep 2009), that K63- and tandem-Ub-chains have the same overall structure but that the chemical environment at the isopeptide bond of K63-linkage is different than that from the peptide bond of C-terminal-N-terminal linkage. Since in Fig. 1 the authors show that the UIM-Ub-binding doesn't involve the isopeptide bond, which is mainly the different feature of K63- and tandem-linked Ub-chains, it needs to be explained how this specificity towards the K63-linked chains excludes the binding to tandem Ub.
3.) Different amino acids are predicted from the structure to be involved in the binding of one UIM to one Ub moiety. To prove this it would be good to mutate some of these residues and to perform binding assays with these mutants.
4.) The suggestion that the linker needs a -helical structure for selective Ub-chain binding could be addressed for instance by exchanging the RAP80-UIM linker with the Epsin UIM1-UIM2 linker, which is expected not to fold to a -helical structure. Additionally, any random sequence with the same length as the RAP80-UIM linker that makes stable -helix could be inserted between the RAP80-UIM and this construct tested for selective binding to different Ub2-chains.
5.) RAP80 localises to gammaH2AX-positive foci at points of DNA-double-strand-breaks (DSBs), which depends on the binding of the UIMs to K63-linked Ub-chains. To address the physiological role of the linker in binding to K63-linked Ub-chains, the authors could induce DNA-double strand breaks (e.g. with Ionizing radiation) and test if different RAP80-constructs containing a linker with which RAP80-UIMs do not bind K63-Ub-chains still can go to foci that mark DSBs. For this experiment constructs with a linker suggested in 4.) and the linkers they used in Fig.4 could be used.

Minor comments:
Fig. 4C, 5b, S2b: The incubation time for these pull-down assays is 5 minutes. It would be good to also test longer incubation times. Also the amount of Ub-chains used for the GST-PD assays should be mentioned, since it is nowhere stated.
Fig. 5b is very similar to fig. 3d of Sims and Cohens paper which makes it kind of redundant, since the main difference is that it shows the sequences of mouse proteins instead of humans as in the paper from Sims and Cohen.

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1st Revision - authors' response 17 May 2009

Comments from Referee #1:

1. The paper needs to be brought up-to-date, especially in the introduction. For example, heterotypic chains also exist and K11 chains are abundant and signal for degradation. See Xu et al. Cell 2009 and Meierhofer et al. J. of Proteome 2008. And, as mentioned above, the Sims and Cohen paper should be referenced.

We updated the introduction and mentioned the role of non-K63 linked chains for the proteasomal degradation (ln 7 ñ 17, pg 3 and ln 22, pg 4 ñ ln 6, pg5). Also, we referenced the papers Referee #1 suggested.

2. The authors make a convincing argument for why Rap80's UIMs require K63 linkages. But, it is not clear why UIM1 binds the proximal subunit while UIM2 binds the distal subunit. Are any other species present? If not, is this singular binding mode an artifact of crystallization? Could the reverse orientation with UIM1 on distal and UIM2 on proximal exist in solution?

Requirement of the inter-UIM region for the di-ubiquitinïUIM1-UIM2 interaction indicates that UIM1 and UIM2 cannot independently bind with distinct ubiquitin chains. Therefore, the binding of UIM1 to distal and that of UIM2 to proximal cannot exist in solution. We mentioned this point in ln 6, pg9 ñ ln8, pg9. The reverse orientation requires the other ubiquitin-binding motif "MIU", which has a reverse ubiquitin-binding motif (amino acid sequences) compared to UIM. Therefore, the reverse orientation with UIM1 on distal and UIM2 on proximal in one RAP80-UIM1-UIM2 molecule cannot exist.

Comments from Referee #2:

1. RAP80 is specific for K63-linked di-Ub and doesn't bind linear diUb, even though the linkage between the two Ub moieties are not involved in binding to RAP80. Komander et al (EMBO Reports) have recently shown that K63-linked and linear di-Ub have very similar structures. Can the authors speculate on the basis for the K63-mediated specificity?

We speculate that the possible C-terminal tail conformation of the distal ubiquitin in linear di-ubiquitin bound to RAP80-UIM1-UIM2 is energetically disadvantageous and/or that van-der-Waals contact between Leu 73 of the distal ubiquitin and the inter-UIM region may restrict the flexibility of the C-terminal tail conformation of the distal ubiquitin. We mentioned these two points in the subsection "Linkage selectivity of RAP80-UIM1-UIM2", ln.9, pg 9 ñ ln 12, pg 10.

2. Recently Rahighi et al (Cell) published the structure of the UBAN domain of NEMO in complex with linear di-Ub. The authors should discuss this work.

We compared our structure with the UBANïdi-Ub complex structure and discussed the mechanism to discriminate Lys 63-linked and linear ubiquitin chains in the subsection "Linkage selectivity of RAP80-UIM1-UIM2", ln.20, pg 9 ñ ln 12, pg 10.

3. I'd recommend that Supp Fig S2 is included in the main manuscript.

Supplementary Fig. S2 was merged with Fig 1. Figs. 1, S2a, and S2b are renamed Figs. 1c, 1a, and 1b, respectively

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