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SAS-6 coiled coil structure and interaction with SAS-5 suggests a regulatory mechanism in C. elegans centriole assembly

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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 29 May 2012

Thank you for submitting your manuscript for consideration by The EMBO Journal, and please excuse the delay in getting back to you with a response, due to a late referee report. We have now received the comments from three experts (copied below), who indicate overall interest in your results and conclusions and would therefore in principle support publication of a revised manuscript in our journal. Nevertheless, they also raise several substantive points that would need to be addressed prior to eventual acceptance. In this respect, one key issue to address will be referee 2's concern with the electron micrographs in Figure 7. It will also be essential to validate the in vitro findings and conclusions by some in vivo or cellular experiments as requested by referee 1; however it should in my opinion suffice to mutationally test the significance of the interactions in either C. elegans (ref 1 point 1) or in any other system bearing conserved interaction residues (ref 1 point 3), but not necessarily in both. The referees also make various mostly well-taken suggestions for revising the presentation and interpretations, which I would ask you to take into account; this may also include moving some more peripheral data into the supplement, but completely removing certain data (as recommended by referee 2) would in my view not be warranted. Finally, please make sure to include the PDB accession codes in the revised manuscript upon resubmission.

Please be reminded that it is our policy to allow a single round of major revision only, and that it will therefore be important to diligently and comprehensively answer to all the specific points raised at this stage in the process. When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the 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

We generally allow three months as standard revision time, and it is our policy that competing

manuscripts published during this period will have no negative impact on our final assessment of your revised study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider this work, and please do not hesitate to contact me in case you should have any additional question regarding this decision or the reports. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

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REFEREE REPORTS:

Referee #1 (Remarks to the Author):

In this manuscript Qiao and colleagues use X-ray crystallography in combination with biochemical/biophysical analyses to study the physical relationship between two core centriole proteins in C. elegans, SAS-5 and SAS-6. These two centriole components are conserved in many centriole-bearing organism including humans and flies and are both required for centriole duplication in the organism so far tested.

Briefly, the authors show that SAS-5 and SAS-6 interact directly through a short coiled coil segment in the C-terminal region of SAS-5 (aa 390-404) and a narrow central region of SAS-6 (aa 390-404). The authors provide compelling evidence that this interaction is mediated by synergistic electrostatic and hydrophobic interactions between specific residues is the two proteins. Furthermore the author show that SAS-6 forms an autoinhibitory tetramer and that binding of the SAS-5 C-terminal domain to the SAS-6 prevents tetrameric association of SAS-6 which would facilitate robust centriole, or central tube, assembly.

Overall this is a well-executed study with very convincing structural/biochemical data. Although it has been previously proposed that SAS-5 and SAS-6 associate the molecular basis for their tight association and cooperation during centriole assembly have remained elusive. This study provides potentially interesting insights on the relationship of these two core centriole proteins during centriole assembly.

Although I am generally supportive of this paper, where it lacks, in this reviewer's opinion, is in supporting their model based on in vitro work with in vivo data. In particular, the authors should consider the following issues:

1- The mutations is SAS-5 and SAS-6 shown to interfere with the interaction between the two proteins should in principle inhibit centriole duplication in vivo. The authors should test this possibility be performing RNAi-rescue experiments in C. elegans.

2- The authors mention that the key residues identified in this study are conserved in several other nematodes. It is not clear if these residues, and or electrostatic distribution of residues is also conserved (or not) in other species (humans, flies, etc.) and also show the same binding properties. Reading the paper it is not very clear if the authors are proposing that this SAS-5 and SAS-6 relationship discussed here is restricted to C. elegans or also applies to other SAS-6 and SAS-5/Ana2/STIL containing organisms. This should be clarified both textually and experimentally. If only restricted to C. elegans, the authors should make this very clear, including in the title.

3- Related to the above points, if the authors think that the assembly principles described here apply to other organism, his should be tested experimentally, for example by also using mutational

analysis in RNAi rescue experiments in human tissue culture cells. This would significantly increase the impact of the nice work described in this manuscript.

Minor points:

- The authors could mention cep135/bld-10 in the introduction. Referencing to key regulators of centriole assembly also seems to be inaccurate/missing.

- In figure 4, it would be better if the mutations were listed in the figure and/or figure legends. They are referenced in the text, but not in the figure or the caption. The sites they target are labelled, but it is not mentioned in the figure what amino acids have been put in to replace the originals. A nomenclature like E276A, for example, would seem more appropriate.

Referee #2 (Remarks to the Author):

There is a reasonable amount of solid and useful data in this paper (all papers are really about the data in them, not necessarily the "story" they purport to tell), but the authors show lots of data somewhat indiscriminately and speculate rather extensively, instead of presenting a tightly and carefully reasoned analysis. The bottom-line conclusions seem to be that (1) the C-terminal residues of SAS-5 interact with a short segment of the SAS-6 coiled coil; (2) each of the components on its own self-associates (probably non-specifically), but formation of the complex solubilizes it (a technical rather than physiological conclusion); (3) when SAS-5 binds SAS-6, the molar ratio is 1 SAS-5 per SAS-6 dimeric coiled-coil; (4) the SAS-5:SAS-6 complex forms a curved structure with a diameter appropriate for the centriole central tube.

The key result that makes the paper interesting is (at least for someone who does not know the centriole literature) Fig. 7C, but the micrograph fails to show SAS-6 rods, so the interpretation in Fig. 8 is impossible to evaluate. Surely the authors can get better micrographs. Rotary shadowing, properly done, can show dimeric coiled-coils (it was introduced, if I recall corretly, by Henry Slayter, to show the tail of myosin). Thus, the most important improvement that might make the work appropriate for EMBO J, when combined with the binding data and the coiled-coil structure, is a proper version of Fig. 7C. Another obvious question, of course, is why were they unable to get a crystal of a piece of SAS-5 bound to the SAS-6 coiled-coil. The point-mutational data are fine, but they don't lead to a conclusive model for the association, so unfortunately they probably should be deleted from the paper. I would also recommend deleting or minimizing the tetramerization of SAS-6 (no evidence that it is functionally interesting) and removing use of "autoinhibited" in describing that species and the aggregate of SAS-5. "Auto-inhibited" implies function, and this reader's guess is that these are just in vitro artifacts (certainly true for the SAS-5 aggregation). So "mutual regulation" should be removed from the title.

In short, if the authors can provide a convincing micrograph for Fig. 7C, accompanied by a reduced set of data on binding and a brief description of the coiled-coil structure and the position at which SAS-5 interacts, then the paper might well be suitable for re-review (assuming that this reviewer is not overlooking relevant aspects of the centriole literature).

Referee #3 (Remarks to the Author):

This manuscript describes results of biochemical and structural analyses of the binding between two centriole proteins, SAS-5 and SAS-6. Pull-down assays using various recombinant protein fragments narrowed down the binding sites to fairly small regions in the C-terminal domain of SAS-5 and in the coiled-coil domain of SAS-6. Furthermore, X-ray crystallography of the SAS-6 coiled-coil domain suggests the structural basis for the 1:1 interaction between SAS-5 and the SAS-6 dimer. The obtained structural model also explains a bipolar form consisting of two SAS-6 dimers, which is thought to be an inactive state of this protein. Importantly, the authors found that complexes of SAS-6 and MBP-tagged SAS-5 are aligned in an arc-like structure, which apparently corresponds to part of the central tube, the core structure of the C. elegans centriole. From these observations, the authors suggest that SAS-5 facilitates dissociation of the inactive SAS-6 tetramer and association into the central tube.

The presented results are novel and the data are of high quality. Although SAS-5 is a C. elegans protein and not conserved in other centriole-bearing organisms, the presented findings and the regulation model of SAS-6 may well be relevant to the centriole assembly mechanism in other organisms. This paper should therefore be of general interest to the wide readership of the EMBO journal, particularly since the function of SAS-6 in centriole formation recently attracts considerable attention. However, there are several points that I would like the authors to consider before this paper is accepted for publication.

1. The authors' model of the SAS-5 function assumes that its binding to the SAS-6 dimer generates a kink in the coiled-coil rod of SAS-6 and this facilitates its spiral assembly (page 10, 15-16, Fig. 8). It is not entirely clear to me, however, how the kink facilitates spiral assembly. SAS-6 dimers with bent rods can be assembled into either a spiral or a flat ring. I would like the authors explain in more detail.

2. The authors spend a large space for discussion about SAS-4 in the text and in the illustration of Fig. 8. However, since this paper presents no data directly related to SAS-4, detailed discussion about its function may be of little value. I would recommend that the authors touch on the SAS-4 function more briefly, perhaps focusing on the possibility that SAS-4 may surround the core of the central tube that consists of the SAS-5/SAS-6 complexes.

3. It seems odd to me that the authors do not explicitly mention in Introduction that SAS-6 protein has been shown to constitute the cartwheel structure in the centrioles of many organisms other than C. elegans. The authors should reference the first paper to show that SAS-6 constitutes the cartwheel and the importance of the cartwheel for the establishment of the 9-fold symmetry (Nakazawa et al., 2007) .

1st Revision - authors' response 28 August 2012

First of all, we would like to thank all three reviewers for their thorough and constructive comments on our manuscript. Following their suggestions, we have extensively reworked our manuscript and include new data verifying our *in vitro* predictions *in vivo* in the *C. elegans* embryo (new Figure 4). We also include new electron micrographs of the SAS-6/SAS-5 CTD complex (revised Figure 5G). Furthermore, we have addressed all conceptual comments, clarifying confusion in the text as suggested or making necessary modifications wherever is necessary. We hope that, with these introduced changes, this manuscript is now suitable for publication in the EMBO Journal. A detailed response is shown below.
General remarks:

1. To make the figures more compact, we have combined previous Fig. 1 and 2 into a single figure (Figure 1). We did the same for Fig. 4 & 5 (new Figure 3), plus moving all ITC data in the figures into Supplementary Figure 4. Similarly, we combined previous Fig. 6 and 7 into new Figure 5.
2. New rotary metal shadowing micrographs of SAS-6 (aa1-410) alone and in complex with the MBP-tagged SAS-5 CTD have been added to Figure 5F & G, respectively.

3. Due to space limitations, the descriptions of ITC, SLS, and all in vivo experiment related information (Material and Methods) have be moved to the Supplementary information.

4. Atomic coordinates and structure factors have been deposited in the PDB with entry codes 4GKW.

Referee #1

In this manuscript Qiao and colleagues use X-ray crystallography in combination with biochemical/biophysical analyses to study the physical relationship between two core centriole proteins in C. elegans, SAS-5 and SAS-6. These two centriole components are conserved in many centriole-bearing organism including humans and flies and are both required for centriole duplication in the organism so far tested.

Briefly, the authors show that SAS-5 and SAS-6 interact directly through a short coiled coil segment in the C-terminal region of SAS-5 (aa 390-404) and a narrow central region of SAS-6 (aa 390-404). The authors provide compelling evidence that this interaction is mediated by synergistic electrostatic and hydrophobic interactions between specific residues is the two proteins. Furthermore the author show that SAS-6 forms an autoinhibitory tetramer and that binding of the SAS-5 C-terminal domain to the SAS-6 prevents tetrameric association of SAS-6 which would facilitate robust centriole, or central tube, assembly.

Overall this is a well-executed study with very convincing structural/biochemical data. Although it has been previously proposed that SAS-5 and SAS-6 associate, the molecular basis for their tight association and cooperation during centriole assembly have remained elusive. This study provides potentially interesting insights on the relationship of these two core centriole proteins during centriole assembly.

Although I am generally supportive of this paper, where it lacks, in this reviewer's opinion, is in supporting their model based on in vitro work with in vivo data. In particular, the authors should consider the following issues:

1- The mutations in SAS-5 and SAS-6 shown to interfere with the interaction between the two proteins should in principle inhibit centriole duplication in vivo. The authors should test this possibility by performing RNAi-rescue experiments in C. elegans.

: We really appreciate the viewer's suggestion for the RNAi-rescue experiments, which would certainly validate and strengthen the in vitro biochemical interaction results. Since previous work has already shown that one of the SAS-5 mutations we mapped to abolish SAS-6 interaction, R397C, led to the mislocalization of SAS-5 and the failure in centriole assembly (Dellattre et al., 2004), we have focused on testing the three SAS-6 mutations that could not interact with SAS-5 in vitro, namely mA, mB and mC. We obtained several independent strains with identical behavior for mA and mC (no transformats were obtained for mB). As shown in Figure 4A, neither mutant could sustain centriole assembly following depletion of the endogenous protein by RNAi. We further used the mating assay to specifically assess SAS-6 recruitment to the procentriole, which shows that SAS-6 recruitment was nearly completely abolished for both mA and mC when endogenous SAS6 was depleted, mirroring the SAS-5 depletion phenotype (Figure 4B). Therefore, the residues mutated in mA and mD are indeed critical for SAS-6 recruitment and function in centriole assembly.

2- The authors mention that the key residues identified in this study are conserved in several other nematodes. It is not clear if these residues, and or electrostatic distribution of residues is also conserved (or not) in other species (humans, flies, etc.) and also show the same binding properties. Reading the paper it is not very clear if the authors are proposing that this SAS-5 and SAS-6 relationship discussed here is restricted to C. elegans or also applies to other SAS-6 and SAS-5/Ana2/STIL containing organisms. This should be clarified both textually and experimentally. If only restricted to C. elegans, the authors should make this very clear, including in the title.

: Following the suggestion, the title has been modified to "SAS-6 coiled coil structure and interaction with SAS-5 suggest a regulatory mechanism in ***C. elegans*** centriole assembly". Given the poor sequence homology between SAS-5 and its putative orthologs Ana2 and STIL the interaction interface we determined for SAS-5/SAS-6 cannot indeed be unquestioningly applied to Ana2/DSas-6 or STIL/hsSAS-6. Instead, as we outline in the Discussion, our work leads to testable predictions for those proteins' binding to SAS-6 in other organisms, which will allow future work to verify that these proteins are indeed distant homologs of C. elegans SAS-5, something that still remains debatable at this point.

3- Related to the above points, if the authors think that the assembly principles described here apply to other organism, this should be tested experimentally, for example by also using mutational analysis in RNAi rescue experiments in human tissue culture cells. This would significantly increase the impact of the nice work described in this manuscript.

: As was answered above, this study is about the interaction and function of SAS-6 and SAS-5 in *C. elegans*. Certainly, it would be important and interesting to investigate in the future whether the cooperative function of SAS-5/SAS-6 in centriole assembly is conserved in other organisms, but this is beyond the scope of this manuscript.

Minor points:

- The authors could mention cep135/bld-10 in the introduction. Referencing to key regulators of centriole assembly also seems to be inaccurate/missing.

: Thanks for pointing this out. Cep135/Bld10 is now also mentioned in the introduction (page 3). References were checked and regulation of SAS-5/ZYG-1 recruitment in centriole assembly by PP2A was added to the introduction (page 3).

- In figure 4, it would be better if the mutations were listed in the figure and/or figure legends. They are referenced in the text, but not in the figure or the caption. The sites they target are labelled, but it is not mentioned in the figure what amino acids have been put in to replace the originals. A nomenclature like E276A, for example, would seem more appropriate.

: Good point. This is now fixed (see Figure 3D).

Referee #2

There is a reasonable amount of solid and useful data in this paper (all papers are really about the data in them, not necessarily the "story" they purport to tell), but the authors show lots of data somewhat indiscriminately and speculate rather extensively, instead of presenting a tightly and carefully reasoned analysis. The bottom-line conclusions seem to be that (1) the C-terminal residues of SAS-5 interact with a short segment of the SAS-6 coiled coil; (2) each of the components on its own self-associates (probably non-specifically), but formation of the complex solubilizes it (a technical rather than physiological conclusion); (3) when SAS-5 binds SAS-6, the molar ratio is 1 SAS-5 per SAS-6 dimeric coiled-coil; (4) the SAS-5:SAS-6 complex forms a curved structure with a diameter appropriate for the centriole central tube.

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In short, if the authors can provide a convincing micrograph for Fig. 7C, accompanied by a reduced set of data on binding and a brief description of the coiled-coil structure and the position at which

SAS-5 interacts, then the paper might well be suitable for re-review (assuming that this reviewer is not overlooking relevant aspects of the centriole literature).

: Regarding the electron micrographs in Figure 7C, as described in the Discussion (page 21-22), using full-length SAS-5 and SAS-6 proteins we observe arc-like structures involving multiple copies of SAS-5/SAS-6. Extrapolating to a closed circle, those circles would have an average diameter of ~63 nm, close to the dimensions of the inner part of the central tube seen in electron micrographs of C. elegans embryos (Pelletier et al., 2006). Remarkably, such circles would be composed of ~8-10 repeated (globular) structures, which is consistent with the characteristic nine-fold symmetry of centrioles. Unfortunately, we have so far been unable to detect such closed circles, which may be due to a lack of post-translational modification, requiring ZYG-1 or other factors (Supplementary Figure 8). While the SAS-6 coiled coil has proven difficult to detect using full-length proteins, new experiments with residues 1-410 of SAS-6 and the SAS-5 CTD show monomeric particles with clearly visible coiled coil domains rather than arc-like structures (compare Figure 5G and Figure 6B); the binding mode of SAS-5 further confirmed our mapped interaction sites on both proteins and indicated that the invisible coiled coil in the full-length complex is not due to a technical problem. We speculate that such phenomenon for the full-length complex might arise from the structural modulation of SAS-6 upon SAS-5 binding, which remains as an open question to be answered in the future.
: Regarding the question "why were they unable to get a crystal of a piece of SAS-5 bound to the SAS-6 coiled-coil", we should admit that we had tried that very hard and have indeed obtained nice-looking crystals for the complex. However, after over a year of efforts, none of the crystals we obtained could diffract beyond 10-Å resolution. That was the reason why we turned to mutagenesis studies to pinpoint the interaction sites on both proteins.
: Although we understand this reviewer's concern about the validity of the mutation data, we do not agree with the reviewer's comment that "the point-mutational data don't lead to a conclusive model for the association". Indeed, a high-resolution structure would be much more informative to explicitly reveal the specific interactions of different components in a protein complex. However, when such a complex structure is not available, people would carry out mutational studies, which at present remain as one of the standard complementary techniques for structural studies of marcromolecular complexes. Of course, all mutational data should be taken with caution without other evidence to back them up. In our case, we have carried out *in vivo* tests of these mutations and the results are in agreement with and strongly support the *in vitro* biochemical data (Figure 4). Therefore, we opt to keep our mutational data in the manuscript.
: Following the reviewer's suggestion, we have removed "mutual regulation" in the title and replaced the "autoinhibitory" term in the text by "oligomeric" (in Abstract and Discussion).

Referee #3

This manuscript describes results of biochemical and structural analyses of the binding between two centriole proteins, SAS-5 and SAS-6. Pull-down assays using various recombinant protein fragments narrowed down the binding sites to fairly small regions in the C-terminal domain of SAS-5 and in the coiled-coil domain of SAS-6. Furthermore, X-ray crystallography of the SAS-6 coiled-coil domain suggests the structural basis for the 1:1 interaction between SAS-5 and the SAS-6 dimer. The obtained structural model also explains a bipolar form consisting of two SAS-6 dimers, which is thought to be an inactive state of this protein. Importantly, the authors found that complexes of SAS-6 and MBP-tagged SAS-5 are aligned in an arc-like structure, which apparently corresponds to part of the central tube, the core structure of the C. elegans centriole. From these observations, the authors suggest that SAS-5 facilitates dissociation of the inactive SAS-6 tetramer and association into the central tube.

The presented results are novel and the data are of high quality. Although SAS-5 is a C. elegans protein and not conserved in other centriole-bearing organisms, the presented findings and the regulation model of SAS-6 may well be relevant to the centriole assembly mechanism in other organisms. This paper should therefore be of general interest to the wide readership of the EMBO journal, particularly since the function of SAS-6 in centriole formation recently attracts considerable attention. However, there are several points that I would like the authors to consider before this paper is accepted for publication.

1. The authors' model of the SAS-5 function assumes that its binding to the SAS-6 dimer generates a kink in the coiled-coil rod of SAS-6 and this facilitates its spiral assembly (page 10, 15-16, Fig. 8). It is not entirely clear to me, however, how the kink facilitates spiral assembly. SAS-6 dimers with bent rods can be assembled into either a spiral or a flat ring. I would like the authors explain in more detail.

: We would like to thank this reviewer for asking this question. In the past a few weeks, we have tried to verify our initial hypothetical model "binding of SAS-5 bends the SAS-6 CCD" employing different experimental techniques, including small angle x-ray scattering (SAXS) and rotary metal shadowing EM. Unfortunately, with the resolution limitation of these techniques, we could not reach such a conclusion. Therefore, we have removed the kinked coiled coil prediction and the spiral assembly proposal in the final working model (Figure 6). We think that the reason for SAS-5 to bind to only one of the two interacting sites on the SAS-6 coiled coil could equally be either that binding of SAS-5 to one site occludes the other site from binding the second SAS-5 or that SAS-5 binding disrupts the structural symmetry by inducing local conformational changes of the SAS-6 coiled coil (see Results – page 10-11; Discussion – page 18, first paragraph).

2. The authors spend a large space for discussion about SAS-4 in the text and in the illustration of Fig. 8. However, since this paper presents no data directly related to SAS-4, detailed discussion about its function may be of little value. I would recommend that the authors touch on the SAS-4 function more briefly, perhaps focusing on the possibility that SAS-4 may surround the core of the central tube that consists of the SAS-5/SAS-6 complexes.

: As suggested, extensive discussion of SAS-4 in centriole assembly has been largely removed, with only a brief mention of its role for the formation of the outer wall of the central tube (page 16-17).

3. It seems odd to me that the authors do not explicitly mention in Introduction that SAS-6 protein has been shown to constitute the cartwheel structure in the centrioles of many organisms other than C. elegans. The authors should reference the first paper to show that SAS-6 constitutes the cartwheel and the importance of the cartwheel for the establishment of the 9-fold symmetry ().

: Thanks for pointing this out. This is now fixed and the paper by Nakazawa et al. has been cited in the manuscript (see the second paragraph on page 2).

Acceptance letter 17 September 2012

Thank you for submitting your revised manuscript for our consideration. It has now been seen once more by the original referees (see comments below), and I am happy to inform you that all of them consider the manuscript significantly improved and therefore now well-suited for publication in The EMBO Journal.

Before proceeding with formal acceptance, I would just like to give you an opportunity to incorporate some answer(s) to the one remaining comment of referee 2 (see below) into the manuscript. The easiest way to do this would be for you to simply send us an appropriately modified manuscript text file by email, so that we could replace it in our system. Should you also want to show an additional (supplementary?) image as recommended by the reviewer, then please also send us either a revised figure or supplementary information file.

Once we will have received these final edits, we shall be happy to formally accept the paper and send it off for production. Thank you once again for the opportunity to consider this work for publication, as well as for your exhaustive and comprehensive efforts to address the reviewers' comments, and congratulations on a successful publication! Please consider us again in the future for your most exciting work.

Yours sincerely,
Editor
The EMBO Journal

Referee #1

(Remarks to the Author)
The authors have done a very good job addressing my initial concerns and those of the other reviewers. I am particularly happy to see the in vivo work showing that the SAS-5/SAS-6 mutants that impair binding prevent normal centriole duplication. The manuscript, from this reviewers perspective, is ready for publication in EMBO.

Referee #2

(Remarks to the Author)
The MS is much improved. The new EM pictures are far clearer than the previous ones.
My only question is why can't you see the putative ring of SAS-6 heads (even if coiled-coil stalks are hard to contrast) in the micrographs in Fig. 6. They all have to be there, because you'd never otherwise get those nice arcs. So, in a minor revision (NOT requiring re-review, please!), could you add to the figure caption an explanation (or attempted explanation) of the answers to my questions above. It would also help to show a large field of a number of such arcs, to convince the reader that the small images are not selected very non-randomly from fields that do not show these arcs in any preponderence.

Referee #3

(Remarks to the Author)
The authors incorporated all of my comments and adequately modified the manuscript. I would like to recommend to publish this paper in EMBO Journal.

Additional corresopndence 19 September 2012

Attached are two files. The doc file is a slightly modified version of the original manuscript, incorporating the suggestion from referee 2. The change is on page 21 and is highlighted in sky blue (two sentences), which tells a similar rod-missing observation for the cross-linked sample of the truncated SAS-5/SAS-6 complex. The pdf file contains all 10 supplementary figures (S1-10), which should replace the previously submitted Figure S1-9. The difference is that we have added an extra Figure S8 containing four large-field EM images for the SAS-5/SAS-6 complex as requested by referee 2. Previous Figure S8 and S9 are now accordingly renamed S9 and S10, respectively.

