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STAG3-mediated stabilization of REC8 cohesin complexes promotes chromosome synapsis during meiosis

Tomoyuki Fukuda, Nanaho Fukuda, Ana Agostinho, Abrahan Hernández-Hernández, Anna Kouznetsova, Christer Höög

Corresponding author: Christer Höög, Dept of Cell and Molecular Biology

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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 December 2013

Thank you for submitting your manuscript on STAG3 roles in mammalian meiosis for our editorial consideration. We have now received the comments of three expert referees, which you will find copied below for your information. As you will see, the referees express interest in principle in this work and its findings, but also indicate a number of points that would need to be followed up on in order to warrant publication in a broad general journal such as this one. Of particular importance in this respect is the unclear relation of STAG3 meiotic roles to STAG1/2, and the missing analysis of STAG1/2 fate and roles in mediating residual cohesion in this case; it would also appear important to reference and discuss reported mouse knock-out work on these STAG3 paralogs.

Should you be able to address these above concerns as well as the various other issues, and to extend the study along the lines suggested by all three referees (which may also require the use of complementary techniques/approaches), we would be open to consider this manuscript further for publication in our journal. I should however point out that it is our policy to allow only a single round of major revision, making it essential to carefully respond to all points raised at this stage. When preparing your letter of response to the referees' comments, please also remember that this will form part of the Review Process File, and will therefore be available online to the community.

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.

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REFEREE REPORTS:

Referee #1:

Cohesion between sister chromatids is maintained by a ring-shaped protein structure, the cohesion complex. The cohesion complex contains four subunits, two SMC proteins, one a-kleisin protein and one SA (stromal antigen) protein, and variations in the composition of the cohesion subunit generate functional diversity. A meiosis-specific SA protein, STAG3, is expressed during vertebrate meiosis. The authors generated mice with a hypomorphic allele of STAG3, and show that the formation of the chromosome axis, homolog synapsis and recombination are severely impaired in these mice, resulting in infertility. Since STAG3 is required for the protein stability of REC8, the authors conclude that the STAG3-REC8 cohesin complex is essential for proper chromosome structure and meiotic progression.
Although STAG3 still remains at low levels in the mutant mouse, the dosage defects are interesting. The following points should be addressed for publication in this journal.

Major comments:
1) The authors reveal that short axial structures are observed in the STAG3 mutant, suggesting that the meiotic chromosome axis can form independently of STAG3 or alternatively dependent on residual levels of STAG3 in their hypomorphic STAG3 mutant. To explore this issue further, immunostaining STAG1/STAG2 should be performed in the mutant.

2) Does SYCP1-labeling represent inter-sister synapsis in STAG3 KO? (Fig5A, B, Page 8).

3) The authors state that the reduced expression of STAG3 in homozygous STAG3 mutant meiocytes does not abolish sister chromatid cohesion because the proper number of centromeres exist in oocytes (Fig5C). However, it is uncertain whether sister chromatid cohesion is generally preserved in other regions (e.g., at chromosome arms). Convincing evidence could be provided if sister chromatid cohesion is examined by FISH at some chromosome arm loci.

4) It would be better to quantify centromere splitting in oocytes (Figure 5). Is this defect also observed in spermatocytes? Do the authors have any idea why some centromeres are split in STAG3 KO? How about telomere splitting?

Minor comments:
1. In Figure 2E, there are two sets of wild-type images. Delete one of them, or make mention of them in the manuscript.

Referee #2:

In the current study from the Hˆˆg lab, mice carrying a hypomorphic allele of the meiosis-specific subunit STAG3 are generated and characterized. These mice are viable but infertile and meiocytes from both sexes display severe defects in synapsis. Although, in principle, the three alpha kleisin subunits present in meiotic cells can form a complex with STAG3, the authors show that REC8 and, to a lesser extent, RAD21L are most affected by the drastic reduction in STAG3 levels.

The characterization of this mouse model confirms the (expected) importance of STAG3 function for meiosis. However, I do not think that this is sufficient to grant publication in EMBO Journal. I do not see a clear conceptual advance in terms of the division of labour among the different versions of cohesin that coexist in meiotic cells. Moreover, the fact that there is still some residual Stag3

prevents strong conclusions to be made, e.g. when short axial structures labeled by RAD21L, RAD21 and AE antibodies are observed in homozygous Stag3 mutant meiocytes, the authors cannot conclude that the meiotic chromosome axis can form independently of STAG3, since it is also possible that the residual STAG3 contributes to axis formation.

In addition, most results in the paper are based on immunofluorescence staining, with the exception of the immunoblotting in Figure 3D/E. I imagine that doing biochemistry in testis extracts is quite difficult but it is important to evaluate the relative abundance of the different cohesin complexes (and how it changes in the absence of Stag3) in order to understand their role in the formation of the SC, etc. The experiments in Figure 3D/E could be extended by using immunoprecipitation (like in Lee & Hirano 2011, JCB) and also by immunoblotting with additional antibodies. Since this study is about STAG3, an obvious question is what happens to STAG1 and STAG2.

Could the authors at least speculate why REC8 loss is larger than the RAD21L loss when the levels of STAG3 are drastically reduced? Maybe STAG1/2 can compensate the loss of STAG3 in RAD21L containing complexes but not in REC8 containing complexes? Maybe the affinity of STAG3 for RAD21L is much higher than for REC8 so that the residual STAG3 forms a complex only with the former?

Referee #3:

Cohesins are essential structural components of the chromosomes essential for correct segregation at cell division. The subunits constituting the cohesion ring vary with some meiotic components overlapping with mitotic components and some being unique. In particular the predominant STAG protein expressed in meiosis is STAG3. This paper reports the effects of a hypermorphic allele of STAG3 on meiosis in mouse. The authors find that the reduced levels of STAG3 in homozygotes for this hypermorph are infertile in both sexes, that axis formation is severely impaired, homologous chromosomes fail to synapse and that, probably as a result of this, DSB repair, recombination and crossover do not complete. The axial structures formed contain the klesins RAD21 and RAD21L but not REC8 which the authors show is depleted by some unknown mechanism in these animals.
The data support the conclusions that the cohesin complex of SMC1b,REC8 and STAG3 support axis formation, with a contribution from the complex containing RAD21L. Overall the phenotype of the STAG3 mutants is very similar to that of REC8, consistent with the loss of this latter protein which is known to cause synapsis between sister chromatids. The speculation that the SMC1b, REC8 and STAG3 complex promotes inter-homologue synapsis is probably justified.
In summary this paper provides convincing evidence for the role of STAG3 in meiotic axis formation and in conjunction with REC8 defines the cohesion complex predominantly responsible for this.

Minor points.
It would be useful to have quantitation of the level of remaining STAG3 mRNA and protein, in the case of the mRNA at qPCR level and ideally at different stages of germ cell development (fractionation in the case of testis, developmental time points for females)
Are the short sections of axis in the mutant indeed shortened axes or are they fragments of axis formed in pericentromeric regions as in Winters et al?
Telomeric staining would be of value to support the claim that chromosome axes are shortened rather than only forming in a fragmented manner near centromeres
Fig1A is oversimplified since STAG1 and 2 are expressed early in meiosis
Fig1B RAD21- and RAD21L-containing "late pachytene" images differ by REC8 and STAG3 staining. If this is due to differences in substaging within late pachytene it should be recognised in the text
Fig 5A seems to have arrow heads in all panels not arrow in middle panel as described
Fig 6E Poor spread for MLH1 in wt spermatocytes could be replaced

Point-by-point response to the referee's comments

Please find enclosed our revised manuscript. We appreciate very much the referee's comments and have modified the manuscript accordingly. Thus, we have included new experiments, revised the text to a significant extent and eliminated errors. We include a point-by-point response to the referee's comments below.

A week ago, two publications emerged analyzing the same homozygous *Stag3* mutant mouse strain as analyzed by us (Caburet et al, (2014) *N Engl J Med* **370:** 943-949; Llano et al, (2014) *Hum Mol Genet, doi:10.1093/hmg/ddu051)*. It was shown in these two studies that a residual axial structure labeled by cohesin complex proteins and SC proteins remain in mutant meiocytes. The authors of the two studies, however, claim that the homozygous *Stag3* mutant mouse model used in their studies represent a complete loss-of-function (null) model, in which meiocytes are deficient for STAG3 expression. This statement is based on Northern blotting analysis of RNA expression and immunofluorescence staining of meiocytes, neither method sufficiently sensitive to exclude a low level of expression of STAG3. In contrast, we show in our manuscript using RT-PCR and Western blotting analysis that a low level of STAG3 expression remains in homozygous *Stag3* mutant meiocytes. The understanding that STAG3 remains to be expressed at a low level in the homozygous *Stag3* mutant mice is of critical importance in order to understand the phenotype and in agreement with the formation of axial structure labeled by cohesin complex proteins and SC proteins in mutant meiocytes. Furthermore, as shown in the accompanying paper by Winter *et al* using a STAG3-deficient mouse model, complete loss of STAG3 expression eliminates formation of the meiotic chromosome axes and the AEs, verifying that STAG3 (but not STAG1 or STAG2) is essential for association of a-kleisins with the meiotic chromosome axes. Our results, together with the complementary results provided by Winter *et al*, therefore give a correct and unique insight into the role provided by STAG3 in cohesion complex formation, axial organization and SC formation.

Referee #1:
Cohesion between sister chromatids is maintained by a ring-shaped protein structure, the cohesion complex. The cohesion complex contains four subunits, two SMC proteins, one a-kleisin protein and one SA (stromal antigen) protein, and variations in the composition of the cohesion subunit generate functional diversity. A meiosis-specific SA protein, STAG3, is expressed during vertebrate meiosis. The authors generated mice with a hypomorphic allele of STAG3, and show that the formation of the chromosome axis, homolog synapsis and recombination are severely impaired in these mice, resulting in infertility. Since STAG3 is required for the protein stability of REC8, the authors conclude that the STAG3-REC8 cohesin complex is essential for proper chromosome structure and meiotic progression.
Although STAG3 still remains at low levels in the mutant mouse, the dosage defects are interesting. The following points should be addressed for publication in this journal.

Major comments:
1) The authors reveal that short axial structures are observed in the STAG3 mutant, suggesting that the meiotic chromosome axis can form independently of STAG3 or alternatively dependent on residual levels of STAG3 in their hypomorphic STAG3 mutant. To explore this issue further, immunostaining STAG1/STAG2 should be performed in the mutant.

Response:
We have, as requested by the referee, carried out STAG1/STAG2 immunostaining of mutant spermatocytes and oocytes. The new results are included in the manuscript (Figure 5A and B; Figure E2A and B). We found neither STAG1 nor STAG2 to be present on the chromosome axis in the mutant.

2) Does SYCP1-labeling represent inter-sister synapsis in STAG3 KO? (Fig5A, B, Page 8).

Response:
The question raised by the referee has been experimentally addressed in several ways. New FISH analysis results further confirmed that homologous alignment/synapsis is impaired in mutant

spermatocytes (Figure E3). Immunostaining using antibodies against central element proteins (SYCE1, SYCE2 and TEX12) showed that these proteins, identical to SYCP1, labeled the unsynapsed chromosomes in mutant meiocytes (new results found in Figure E4B, C and D). This suggests that transverse filament proteins and CE proteins together form a central region-like structure in association with the axial structures formed in mutant meiocytes. We have now also by super-resolution structured illumination microscopy (SIM) shown that SYCE1 assembles in between the lateral elements (labeled by SYCP3), generating a tripartite structure in homozygous *Stag3* mutant spermatocytes (Figure 6C and Figure E5). Thus, we conclude that inter-sister synapsis occurs in mutant cells.

3) The authors state that the reduced expression of STAG3 in homozygous STAG3 mutant meiocytes does not abolish sister chromatid cohesion because the proper number of centromeres exist in oocytes (Fig5C). However, it is uncertain whether sister chromatid cohesion is generally preserved in other regions (e.g., at chromosome arms). Convincing evidence could be provided if sister chromatid cohesion is examined by FISH at some chromosome arm loci.

Response:
We have added FISH analysis to the manuscript to address the question raised by the referee. We have used a paint probe for chromosome 17 (Figure E3A) and a point probe for the X chromosome (Figure E3B). We found the sister chromatids to be kept together in mutant spermatocytes and oocytes.

4) It would be better to quantify centromere splitting in oocytes (Figure 5). Is this defect also observed in spermatocytes? Do the authors have any idea why some centromeres are split in STAG3 KO? How about telomere splitting?

Response:
We have as suggested by the referee, quantified centromere splitting in oocytes by monitoring ACA staining. We found that homozygous mutant oocytes (E18.5) displayed on average 2.1 chromosomes per cell that showed centromere splitting. Splitting of centromeres was also observed by SIM analysis in spermatocytes (Figure 6C). We do not know why cohesion appears to be reduced at the centromeres in a subset of chromosomes present in mutant meiocytes. We know that while STAG3-REC8 complexes are lost from chromosomes, STAG3-RAD21L and STAG3-RAD21 complexes are retained. The minor cohesion defect observed in the *Stag3* mutant show that REC8 is not critical for retaining cohesion between sister chromatids, but that REC8 has a more pronounced role in holding centromeres together. The antibody against TRF1 (a telomere marker), while useful for identifying chromosome ends, generated a rather diffuse staining pattern not suitable for scoring telomere splitting.

Minor comments:
1. In Figure 2E, there are two sets of wild-type images. Delete one of them, or make mention of them in the manuscript.

Response:
One copy of the wild-type images has been deleted as requested. We have instead added data for oocytes (Figure 2E).

Referee #2:
In the current study from the Hoog lab, mice carrying a hypomorphic allele of the meiosis-specific subunit STAG3 are generated and characterized. These mice are viable but infertile and meiocytes from both sexes display severe defects in synapsis. Although, in principle, the three alpha kleisin subunits present in meiotic cells can form a complex with STAG3, the authors show that REC8 and, to a lesser extent, RAD21L are most affected by the drastic reduction in STAG3 levels.

The characterization of this mouse model confirms the (expected) importance of STAG3 function for meiosis. However, I do not think that this is sufficient to grant publication in EMBO Journal. I do not see a clear conceptual advance in terms of the division of labour among the different versions of cohesin that coexist in meiotic cells. Moreover, the fact that there is still some residual Stag3

prevents strong conclusions to be made, e.g. when short axial structures labeled by RAD21L, RAD21 and AE antibodies are observed in homozygous Stag3 mutant meiocytes, the authors cannot conclude that the meiotic chromosome axis can form independently of STAG3, since it is also possible that the residual STAG3 contributes to axis formation.

Response:
We have analyzed if STAG1 or STAG2 could compensate for reduced STAG3 expression in mutant meiocytes and promote axis formation. We have not been able to detect STAG1 or STAG2 labeling on axial structures in mutant meiocytes (Figure 5A and B), furthermore immunoprecipitation experiments do not show co-precipitation of STAG1 or STAG2 with meiotic complex proteins (Figure 5C and D). Based on these results, it is unlikely that STAG1 or STAG2 contribute to axial formation in the STAG3 mutant. Instead, it is likely that residual STAG3 contributes to axis formation in mutant meiocytes. This model is validated in an accompanying paper by Winter *et al* who have, in contrast to us, analyzed a null *Stag3* mutant mouse model. Winter *et al* found that in the absence of STAG3 expression, axial structures in mutant meiocytes do no form, showing that STAG3 is essential for meiotic chromosome axis formation. We have included this information in the manuscript (Page 15-16). We would argue that the results provided in our manuscript provide novel insights into the dosage effects of STAG3 and identifies a special relationship between STAG3 and REC8, results that are unexpected and intriguing. Such a phenotype has not been reported in other studies analyzing *Smc1b*, *Rec8* or *Rad21l* KO mice. Our results, together with the complementary results provided by Winter et al, give a unique insight into the role provided by STAG3 in cohesion complex formation, axial organization and SC formation, information that we strongly believe would be of interest to the scientific community that follows publications that appear in EMBO J.

In addition, most results in the paper are based on immunofluorescence staining, with the exception of the immunoblotting in Figure 3D/E. I imagine that doing biochemistry in testis extracts is quite difficult but it is important to evaluate the relative abundance of the different cohesin complexes (and how it changes in the absence of Stag3) in order to understand their role in the formation of the SC, etc. The experiments in Figure 3D/E could be extended by using immunoprecipitation (like in Lee & Hirano 2011, JCB) and also by immunoblotting with additional antibodies. Since this study is about STAG3, an obvious question is what happens to STAG1 and STAG2.

Response:
We have analyzed the composition of the meiotic cohesion complexes present in mutant spermatocytes by immunoprecipitation analysis, using anti-RAD21L and anti-SMC1β antibodies (Figure 5C and D). We found the REC8 level to be severely reduced, that the (nuclear) RAD21L level was moderately reduced and that the level of RAD21 was not affected. Immunoblotting data for SMC1α, STAG1 and STAG2 (Figure 5C and D) have now also been included. Furthermore, we have analyzed the protein levels for STAG1 and STAG2 in nuclear extracts from wild-type and mutant testis. No increase in the levels of STAG1 or STAG2 in mutant spermatocytes was found (Figure 5C and D). We also show that neither STAG1 nor STAG2 are co-immunoprecipitated in mutant extracts using anti-RAD21L or anti-SMC1β antibodies (Figure 5 C and D). Furthermore, neither STAG1 nor STAG2 were found to be present on the chromosome axis in the mutant (Figure 5A and B; Figure E2A and B). We therefore conclude that neither STAG1 nor STAG2 become recruited in a compensatory manner to the meiotic chromosome axis in mutant meiocytes.

Could the authors at least speculate why REC8 loss is larger than the RAD21L loss when the levels of STAG3 are drastically reduced? Maybe STAG1/2 can compensate the loss of STAG3 in RAD21L containing complexes but not in REC8 containing complexes? Maybe the affinity of STAG3 for RAD21L is much higher than for REC8 so that the residual STAG3 forms a complex only with the former?

Response:
We have, as discussed in our answer to the previous question, analyzed if STAG1/2 could act in a compensatory manner in mutant meiocytes. We found the immunoblotting levels of STAG1 and STAG2 to be the same in wild-type and mutant spermatocytes and that none of the two proteins was co-immunoprecipitated by RAD21L in the mutant extracts (Figure 5C). Furthermore, neither STAG1 nor STAG2 were found to be present on the chromosome axis in mutant meiocytes (Figure 5A and B; Figure E2A and B). We therefore conclude that neither STAG1 nor STAG2 become

recruited in a compensatory manner to the meiotic chromosome axis in mutant meiocytes. Instead, as suggested by the referee, it is very likely that the explanation lies in the relative affinity of STAG3 for RAD21L vs REC8. The reduced level of STAG3 could also affect the stability of REC8, explaining why REC8 is not only displaced from the chromosomes axis in the mutant, but why the REC8 level in protein extracts are severely reduced. We have added these suggestions to the Discussion of the manuscript (Page 15-16).

Referee #3:

Cohesins are essential structural components of the chromosomes essential for correct segregation at cell division. The subunits constituting the cohesion ring vary with some meiotic components overlapping with mitotic components and some being unique. In particular the predominant STAG protein expressed in meiosis is STAG3. This paper reports the effects of a hypermorphic allele of STAG3 on meiosis in mouse. The authors find that the reduced levels of STAG3 in homozygotes for this hypermorph are infertile in both sexes, that axis formation is severely impaired, homologous chromosomes fail to synapse and that, probably as a result of this, DSB repair, recombination and crossover do not complete. The axial structures formed contain the klesins RAD21 and RAD21L but not REC8 which the authors show is depleted by some unknown mechanism in these animals.
The data support the conclusions that the cohesin complex of SMC1b, REC8 and STAG3 support axis formation, with a contribution from the complex containing RAD21L. Overall the phenotype of the STAG3 mutants is very similar to that of REC8, consistent with the loss of this latter protein which is known to cause synapsis between sister chromatids. The speculation that the SMC1b, REC8 and STAG3 complex promotes inter-homologue synapsis is probably justified.
In summary this paper provides convincing evidence for the role of STAG3 in meiotic axis formation and in conjunction with REC8 defines the cohesion complex predominantly responsible for this.

Minor points.
It would be useful to have quantitation of the level of remaining STAG3 mRNA and protein, in the case of the mRNA at qPCR level and ideally at different stages of germ cell development (fractionation in the case of testis, developmental time points for females)

Response:
We have now quantified the values for the RT-PCR and immunoblotting experiments to estimate the relative levels of STAG3 mRNA and protein levels in spermatocytes in wild-type and mutant spermatocytes (Figure 2C and D). We have also estimated the relative STAG3 levels in juvenile male testes (Figure E1A and B) and in embryonic ovaries (Figure E1C).

Are the short sections of axis in the mutant indeed shortened axes or are they fragments of axis formed in pericentromeric regions as in Winters et al?
Telomeric staining would be of value to support the claim that chromosome axes are shortened rather than only forming in a fragmented manner near centromeres

Response:
We have immunostained mutant spermatocytes and oocytes using a telomere marker (TRF1). We detected a telomere signal at both chromosomal ends. The data has been included in Figure E4.

Fig1A is oversimplified since STAG1 and 2 are expressed early in meiosis

Response:
Figure 1A has been revised as suggested by the referee. STAG1 and STAG2 have now been included as mitotic/meiotic cohesion complex proteins.

Fig1B RAD21- and RAD21L-containing "late pachytene" images differ by REC8 and STAG3 staining. If this is due to differences in substaging within late pachytene it should be recognised in the text

Response:

The comment of the referee is correct; the images represent different substages of pachytene. We have now added this information to the legend of Figure 1B.

Fig 5A seems to have arrow heads in all panels not arrow in middle panel as described

Response:
We thank the referee for pointing out this mistake. We have changed arrows into arrowheads.

Fig 6E Poor spread for MLH1 in wt spermatocytes could be replaced

Response:
New MLH1 data has been added to Figure 7E.

2nd Editorial Decision 17 March 2014

Thank you for submitting your revised manuscript on STAG3 hypomorphic mutant effects on gametogenesis to our editorial office. It has now been assessed once more by two of the original referees, and I am pleased to inform you that they both consider the study significantly improved and now in principle suitable for publication in The EMBO Journal, after modification of some minor presentation-related points (see reports below). I am therefore returning the study to you for one final round of revision, in order to allow you to incorporate these last changes.

Please use the link below to upload this final revision - we should then be able to swiftly proceed with formal acceptance and production of the manuscript!

_____

REFEREE REPORTS:

Referee #1:

The authors properly addressed all my concerns so that this paper should be published.

Referee #2:

I wish to congratulate the authors. The new version of the manuscript is much improved and I think it can now be published in EMBO Journal.

There are just some minor details that I would like to see corrected:

1.In the last paragraph of page 8, twice, "meiotic cohesion complexes" is incorrect, it should be "meiotic cohesin complexes".

2.In the last paragraph of page 14 (in Discussion) the follwing sentence does not make sense:

The changes of the temporal and spatial distribution of STAG3 that occur along the meiotic chromosome axis during the prophase I stage (Figure 1), strongly supporting the notion that STAG3 interacts with the three different -kleisin subunits present in mammalian meiotic cells (Ishiguro et al, 2011; Lee & Hirano, 2011).

Do authors mean "strongly support" or is there something else missing in the sentence?

3. In the western blots on Figure 3D and 5C-D, RAD21 migrates way below 97 kDa. I do not think this is correct. Even though the MW of the RAD21 protein is around 72 kDa, it usually migrates around 120 kDa. Please check out that the band that you are cropping is the correct one or that the size markers are properly labeled.

2nd Revision - authors' response 26 March 2014

Point-by-point response to the referee's comments

Please find enclosed our modifications to our previously revised manuscript. We are grateful that the reviewers found the manuscript significantly improved and now in principle suitable for publication. We include a point-by-point response to the referee's comments below.

Editorial requests:
- Figure Source Data have been added for gels, blots and autoradiographs (Figure 2, Figure 3, Figure 5 and Figure S1).

Referee #2:
1.In the last paragraph of page 8, twice, "meiotic cohesion complexes" is incorrect, it should be "meiotic cohesin complexes".

Response:
We thank the referee for pointing out this error. We have changed the text accordingly.

2.In the last paragraph of page 14 (in Discussion) the follwing sentence does not make sense:
The changes of the temporal and spatial distribution of STAG3 that occur along the meiotic chromosome axis during the prophase I stage (Figure 1), strongly supporting the notion that STAG3 interacts with the three different α-kleisin subunits present in mammalian meiotic cells (Ishiguro et al, 2011; Lee & Hirano, 2011).
Do authors mean "strongly support" or is there something else missing in the sentence?

Response:
We thank the referee for noting this mistake. We have changed the text to "strongly support".

3. In the western blots on Figure 3D and 5C-D, RAD21 migrates way below 97 kDa. I do not think this is correct. Even though the MW of the RAD21 protein is around 72 kDa, it usually migrates around 120 kDa. Please check out that the band that you are cropping is the correct one or that the size markers are properly labeled.

Response:
Migration of RAD21 on the gel is most likely a result of the electrophoresis condition employed; we used a Bis-Tris gel system with MOPS running buffer (Invitrogen), instead of the conventional Laemmli system, using a Tris-glycine running buffer. We have included this information in the Materials and methods section of the manuscript.

