Journal of Cell Science

Dynamic configurations of meiotic DNA-break hotspot determinant proteins

Yu-Chien Chuang and Gerald R. Smith
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MS ID#	Rev#1	Rev#2	Rev#3	Rev#4
JCS258770 (08/06/21)	A	B	C	-
JCS259061 (10/08/21)	A	D	C	B
JCS259061 (05/10/21)	A	D	C	B

Original Submission

Cover letter (9 April 2021)

Dear Dr. Way,

Yu-Chien Chuang and I are submitting for publication in the Journal of Cell Science our manuscript "**Dynamic configurations of meiotic DNA-break hotspot determinants.**" In this paper, we analyze in live cells the S. pombe linear element proteins (LinEs), which bind meiotic chromosomes to guide generation of crossovers necessary for viable gamete formation. We present evidence that these linear elements are more closely related to the well-studied synaptonemal complex of other species than previously recognized.

Our data also indicate that the LinEs have a function beyond their well-established role of binding to DSB hotspots with high specificity and promoting DSB formation there. We have studied live homothallic (wild-type) cells at 25°C to establish meiotic chromosome behavior under optimal conditions. We then compare these results with those using other methods and conditions often used for studying S. pombe meiosis. Our results allow us to clarify seemingly disparate results in the literature. We think our paper will be of major interest to researchers studying meiosis, genetic recombination, and chromosome structure, an area of biology critical for all sexual reproduction.

In our submission on the Journal of Cell Science website we suggest editors and reviewers of our manuscript. We wish to exclude two reviewers (Alexander Lorenz and Daqiao Ding) with whom we have had disagreement and competition.

Thank you for considering our paper for publication in the Journal of Cell Science.

Yours sincerely,
Gerald R. Smith
Member, Division of Basic Sciences

Decision letter (8 June 2021)

MS ID#: JOCES/2021/258770
MS TITLE: Dynamic configurations of meiotic DNA-break hotspot determinants
AUTHORS: Yu-Chien Chuang and Gerald Smith
ARTICLE TYPE: Research Article

Dear Dr. Smith,

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to:

https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area.

(Corresponding author only has access)

As you will see from their reports, both reviewers raise a number of substantial criticisms that prevent me from accepting your paper for publication.

I am very sorry to give you such disappointing news, but we are currently under great pressure for space and it takes a very enthusiastic recommendation by the referees for a manuscript to be accepted.

I do hope you find the comments of the reviewers helpful in allowing you to revise the manuscript for submission elsewhere, and many thanks for sending your work to Journal of Cell Science.

Reviewer 1

Advance summary and potential significance to field

Chuang & Smith, Dynamic configurations of meiotic DNA-break hotspot determinants. JCS_JOCES2021258770

In this manuscript, the authors study the formation of linear elements in fission yeast meiosis, in zygotic and azygotic meiosis, using super-resolution fluorescence microscopy. The authors clearly show that in fission yeast, LinEs form dot-like structures, then change to linear structures to finally go back to dot-like before disappearing at the onset of meiosis I. Thus, for the first time it is shown that pombe LinEs could be more similar to SCs than previously thought. The authors show that indeed this is true, since this linear structures are sensitive to 1,6-hexanediol and to temperature (this is the main reason that has caused this SC-like structures not being observed before in fission yeast meiosis: synchronous meiosis normally are done using a ts allele of the pat1 kinase). In general terms, we have found the manuscript of quality enough to be published in JCS, although there are some minor problems that should be easily corrected by the authors

Comments for the author

My main comment is regarding the consequences of the observation reported here, other than showing, as the authors do, that fission yeast forms synaptonemal complex. Interestingly, the authors show that this structure is temperature sensitive (that is one of the reasons why it was not detected previously). Which are the effects of doing zygotic meiosis at 25ºC vs 34ºC? If there is any effect on spore viability, recombination etc, could it be a consequence of failing to form proper SCs? Authors could further discuss this in the manuscript.

Minor comments:

1.The images of Rec8-mCherry (Fig 5) are not good enough, probably because the authors want to combine GFP of the different LinEs and Cherry from Rec8; if the authors cannot get good imaging from Cherry, then should use Rec8-GFP in parallel experiments
2.Fig S10 is not well described in the figure legend. Which are untreated and which are treated samples?
3.In the references, Martin-Castellanos is misspelled (Mart N-Castellanos)

Reviewer 2

Advance summary and potential significance to field

his work describes microscopic observations of several meiotic proteins in the fission yeast Schizosaccharomyces pombe. Like other organisms, meiotic chromosomes in S. pombe are remodeled around an "axis" formed by meiotic cohesins, which recruit additional regulatory proteins important for pairing of homologues, as well as the formation and repair of meiotic double-strand breaks (DSBs). In many organisms, the axes and associated proteins, which are known as "linear elements" (LinEs) in S. pombe, are held in alignment through formation of the synaptonemal complex (SC), and are sometimes considered to be the "lateral elements" of the SC. Here, Chuang and Smith use high-resolution microscopy, including structured illumination microscopy (SIM) to examine GFP-tagged strains of S. pombe. Specifically, they characterize the physical distribution of a number of LinE proteins as well as the cohesin subunit Rec8. In agreement with previous work, LinE proteins appear as dot-like structures during early and late prophase, but during the "horsetail stage" in which the nuclei undergo extensive telomere-driven motion, they appear as short linear fragments that do not extend along the full length of chromosomes, consistent with the idea that LinEs may be discontinuous. Recent studies from other groups have reported very similar observations; indeed I was unable to determine what aspects of this manuscript are novel, other than the finding that some LinE structures appear to be sensitive to hexanediol, a result I find questionable (see below). In my view, the transition from dot-like to linear structures likely reflects the highly elongated chromosome configuration present during the horsetail stage, rather than a functional difference in association of LinE proteins.

Comments for the author

I find the presentation of the work to focus inordinately on prior results and interpretations from the authors' group while ignoring other relevant published data. In particular, I do not agree with the foundational statement in this manuscript that "LinE proteins are distantly related to the synaptonemal complex (SC) proteins of other species." LinE proteins are related to chromosome axis proteins in other species, but this is a structure distinct from the SC central region. SCs have been found to be absent in S. pombe as well as a few other fungi and other cases, such as Drosophila spermatocytes. One LinE protein, Rec27, shows BLAST homology to a central region protein from C. elegans (SYP-2), but this is almost certainly spurious, stemming from the fact that both are coiled-coil proteins, since an evolutionary relationship between these proteins is not supported by phylogenetic analysis.

Perhaps more egregiously, the work fails to cite a recent paper from Ding et al. (https://doi.org/10.1007/s00412-021-00757-w), which included data that overlap extensively with the results presented here, as well as some contradictory findings. In particular, both this manuscript and the published paper also show that LinE proteins form both dot-like and linear

structures, the latter being the dominant form during the horsetail stage. They also both demonstrate that the LinE proteins other than Rec10 are interdependent for their association with meiotic chromosomes, and that Rec10 is required "upstream" of all other LinE proteins. However, in contrast to the current manuscript, Ding et al. concluded that LinE structures are stable and not sensitive to disruption by hexanediol. Clearly, this paper must be cited and the differences in the observations should be directly addressed and ideally explained in a convincing way. It seems unlikely that strain differences or culture conditions would have such a marked effect on the hexanediol sensitivity of these structures. I find the results of Ding et al. somewhat more convincing for 2 reasons: the conclusion that the LinE proteins are insensitive to hexanediol were corroborated by photobleaching experiments that also indicated that LinE proteins are stably bound (i.e., they do not show rapid recovery in FRAP analysis), and also because the hexanediol was added and washed out while imaging. Most cellular assemblies that are sensitive to hexanediol can rapidly reassemble upon removal of the solvent, so this should be tested for the LinE proteins. This is important because extended exposure to hexanediol likely kills cells, making it difficult to conclude that disruption of the LinE structures is biologically meaningful.

All tagged strains should be quantitatively validated by characterizing the duration of meiosis, the crossover distribution, and spore viability.

As far as I am aware, the Smith lab is the only group that considers S. pombe to show crossover interference. The formal definition of CO interference is that crossovers in two intervals on the same chromosome quantitatively "interfere" with each other - that is, the probability of observing a CO in a particular interval is lower if there there is a crossover in a nearby interval along the same chromosome. This has been tested in S. pombe and found not to be the case (https://doi.org/10.1093/genetics/137.3.701). The model previously proposed by Fowler et al. (2018) seems to equate DSB interference with crossover interference. I feel that the authors have cherry-picked results from previous papers that support equating DSB interference with CO interference while ignoring much more compelling evidence that these are separate phenomena - e.g., that CO interference still functions when DSB interference is disrupted, or if breaks are induced by irradiation or DNA-damaging agents. S. pombe also lacks synaptonemal complexes (SCs), and mounting evidence indicates that this structure is essential for crossover interference, although this remains controversial. Further evidence against CO interference is that the pathway that gives rise to COs in S. pombe is different from the ZMM pathway that is subject to interference in other organisms. The concept of CO interference is not necessarily related to the work presented here, but the framing of the work in this way seems misguided to me, and I believe most researchers in the field would share this view.

While some organisms have true DSB "hotspots," in others (e.g., Drosophila, C. elegans, Arabidopsis) there are merely "warm" and cooler regions. DSB maps have now been generated for considerably more organisms than cited here, including several mammals and plants.

The idea that the "dotty" LinE structures reflect clustering of potential sites to mediate DSB interference was presented in previous work from the group and is reiterated here as a model to explain/interpret the observations. However, I don't think that the data in this manuscript provide any support for this notion, particularly for the idea that the dots somehow represent a structure that mediates DSB interference. While the duration of linear LinE structures is modulated by the absence of DSBs, this likely reflects alterations in meiotic progression and the length of the horsetail stage rather than a direct consequence.

I'm not sure what the authors mean by: "The topoisomerase-like protein Spo11, first identified in budding yeast, contains the active site for meiotic DSB formation in multiple experimental organisms" (lines 41-42). While strictly true, this is an awkward way to describe Spo11's role. Additionally, the correlation between binding of LinE proteins and DSB hotspots seems as likely to reflect a role for LinE proteins in repair of DSBs as it does a causal role in DSB formation. The manuscript also seems to ignore data linking transcriptional activity to DSB formation in S. pombe (https://doi.org/10.1534/genetics.116.197954).

I don't understand why the authors conclude that the LinE proteins form a complex in the cytoplasm. Rec25, Rec27, and Mug20 are all very small proteins (<20 kD) and would thus readily pass through nuclear pores without a requirement for an NLS. Also, why would LinE structures differ in zygotic vs. azygotic meiosis?

Ultimately, I find this work to be of insufficient novely or impact for publication in its current form. The discrepancies between these findings and the published results of Ding et al. (2021 Chromosoma) should be addressed before this manuscript is reconsidered.

Reviewer 3

Advance summary and potential significance to field

This is a detailed study from the Smith lab using live-cell microscopy for an exhaustive re-examination of the localization of element proteins in fission yeast and their inter-dependence. S. pombe has typically been reported not to have a synaptonemal complex; this study uses microscopy to argue that there are indeed linear elements that appear to have structures similar to an SC, agreeing with recently published work. The authors also use mutations in different components to perturb the structures and determine inter-dependence. Finally, they compare a normal zygotic meiosis to a manipulated azygotic meiosis (using pat1-ts, which has been heavily used in the field in earlier days) to compare the behavior of the proteins under these different conditions. The use of pat1ts can impose artifacts on meiosis so it is helpful to have a direct comparison.

Comments for the author

This is a descriptive study that relies entirely on microscopic observations, that is of particular interest to the afficionado. It confirms and also expands prior observations from more technically-challenged times.

Concerns are generally minor.
1) Although the abstract states "Here, we investigated LinE configurations and 15 distributions in live cells using super-resolution fluorescence microscopy" only one figure in the paper appears to use SIM, although it is prominently featured in the supplemental. Authors should ensure that each figure indicates the modality employed for imaging. Fig S8 should be moved to the main figures.

2) Rec8 is reported to be resistant to Hexane-diol but there certainly appears to be a reduction in signal lin the Rec10GFP-Hex sample (Fig 5, col 2 row 2). It is not clear that this figure makes an essential contribution, and if it is kept, it should include the quantitation in S11. The additional related data in S10 is also poorly labeled and described. Are these the same cells imaged at 0 and 5'? Or different cells? What's the conclusion? It appears presented because it correlates with observations in other species.

3) The authors should leave the azygotic pat1 meiosis data (e.g., Fig 2) in the supplemental and show that data on zygotic meiosis (e.g., Fig S9). Jumping from one to the other is disorienting to the reader. They can explain that the highly quantitative data are derived from the azygotic.

4) The authors are missing two very relevant recent citations, and should discuss how these fit with their observations and model.
- Ding et al Chromosoma. 2021 Apr 7. doi: 10.1007/s00412-021-00757-w. Linear elements are stable structures along the chromosome axis in fission yeast meiosis

- Escorcia et al Open Biol. 2021 Feb;11(2):200357. doi: 10.1098/rsob.200357. Epub 2021 Feb 24. A visual atlas of meiotic protein dynamics in living fission yeast

Author letter to the editor (9 June 2021)

Dear Dr. Glover,

Thank you for overseeing the review of our manuscript "Dynamic configurations of meiotic DNA-break hotspot determinants" submitted to the *Journal of Cell Science*. To aid our rewriting this manuscript, we would appreciate the following information from Reviewer 2.

1. Are the lateral elements part of the synaptonemal complex? We have assumed so, but the reviewer's Advance Summary suggests a different definition.

2. We would appreciate references for the following statements in Comments for the Author:

a) In paragraph 1: "other relevant published data" (and "Recent studies from other groups" in the Advance Summary). We did not cite Ding et al. because it was not published before we began submitting our manuscript. If this is the only missing citation, nothing further is needed here.

b) Also, in paragraph 1: "not supported by phylogenetic analysis." Is this published? If so, we would appreciate receiving citations for this and the following points.

c) In paragraph 4: "I feel that the authors have cherry-picked results from previous papers that support equating DSB interference with CO interference while ignoring much more compelling evidence that these are separate phenomena - e.g., that CO interference still functions when DSB interference is disrupted, or if breaks are induced by irradiation or DNA-damaging agents."

d) In paragraph 5: "DSB maps have now been generated for considerably more organisms than cited here, including several mammals and plants." Our cited review (Nambiar, Chuang, and Smith 2019) describes genome-wide DSB patterns in budding yeast, fission yeast, Arabidopsis, and mice, but we are unaware of such studies in other species.
We would greatly appreciate your help on this as we continue our research. Thank you.

Yours sincerely,

Gerald R. Smith
Professor, Division of Basic Sciences

Note: There was no reply to the above letter.

Resubmission

Cover Letter (22 June 2021)

Dear Dr. Glover,

Yu-Chien Chuang and I have revised our manuscript "**Dynamic configurations of meiotic DNA-break hotspot determinants**" (MS ID#: JOCES/2021/258770) and resubmitted it to the Journal of Cell Science. We would appreciate your reading our response to the reviews, and our comments below, and reconsidering our manuscript for publication. As requested by Reviewers 2 and 3, we have added citation and discussion of Ding et al. (Chromosoma 2021), which was published just as we were submitting our paper to the Journal of Cell Science.

Reviewers 1 and 3 were quite positive about the substance and value of our research. We have made the minor changes they suggested.

Reviewer 2 was less positive, but we think this reviewer did not consider all the available information in making her or his evaluation. In particular, our previous work on crossover interference was misunderstood. Our results on disruption of linear elements by hexanediol are

consistent with those of Ding et al.; we think Ding et al. misinterpreted their data, which were not quantified. Reviewer 2 mentioned other points in which she or he thinks our conclusions differ from published work. On several of these points, we do not know the publications that Reviewer 2 has in mind, and we have not received a list of them. Thus, we are unable to respond to these points, which we have noted in our response to the reviews.

We would greatly appreciate your considering further our work for publication the Journal of Cell Science. Thank you for your help in making our research available to the community.

Yours sincerely,

Gerald R. Smith

Professor, Division of Basic Sciences

Author response to reviewers' comments

Below, we have **put in bold the many positive comments** by the reviewers, for which we are grateful. Our responses to other points are in red font.

Reviewer 1 Advance Summary and Potential Significance to Field:

In this manuscript, the authors study the formation of linear elements in fission yeast meiosis, in zygotic and azygotic meiosis, using super-resolution fluorescence microscopy. The authors clearly show that in fission yeast, LinEs form dot-like structures, then change to linear structures to finally go back to dot-like before disappearing at the onset of meiosis I. **Thus, for the first time it is shown that pombe LinEs could be more similar to SCs than previously thought**. The authors show that indeed this is true, since this **linear structures are sensitive to 1,6-hexanediol** and to temperature (this is the main reason that has caused this SC-like structures not being observed before in fission yeast meiosis: synchronous meiosis normally are done using a ts allele of the pat1 kinase). In general terms, **we have found the manuscript of quality enough to be published in JCS**, although there are some minor problems that should be easily corrected by the authors

Reviewer 1 Comments for the Author:
My main comment is regarding the consequences of the observation reported here, other than **showing, as the authors do, that fission yeast forms synaptonemal complex. Interestingly, the authors show that this structure is temperature sensitive** (that is one of the reasons why it was not detected previously). Which are the effects of doing zygotic meiosis at 25ºC vs 34ºC? If there is any effect on spore viability, recombination etc, could it be a consequence of failing to form proper SCs? Authors could further discuss this in the manuscript.

We now cite on page 12 previous work showing that spore viability and recombinant frequencies are low above a critical temperature (about 35ºC). This is one reason for using 25ºC as we advocate. We cannot, however, attribute the low recombination or viability at high temperature to defects in the LinEs, for many processes are needed for successful meiosis.

Minor comments:
1. The images of Rec8-mCherry (Fig 5) are not good enough, probably because the authors want to combine GFP of the different LinEs and Cherry from Rec8; if the authors cannot get good imaging from Cherry, then should use Rec8-GFP in parallel experiments

We have replaced the image of Rec8-mCherry in the Rec10-GFP cell (top line) with a more representative figure, in which it is clear that Rec8-mCherry is hexanediol-resistant, as it is in the other cells shown.

2. Fig S10 is not well described in the figure legend. Which are untreated and which are treated samples?

We have relabeled the figure and rewritten the legend to describe more clearly that the images are of multiple cells treated with hexanediol. Each cell is shown by two views – left is GFP (to show the LinE) and right is GFP plus Hoechst (merge; to show LinE co-localization with chromatin). Cells with and cells without hexanediol treatment are in Figure 5.

3. In the references, Martin-Castellanos is misspelled (Mart N-Castellanos)

We have corrected this error.

Reviewer 2 Advance Summary and Potential Significance to Field:
his work describes microscopic observations of several meiotic proteins in the fission yeast Schizosaccharomyces pombe. Like other organisms, meiotic chromosomes in S. pombe are remodeled around an “axis” formed by meiotic cohesins, which recruit additional regulatory proteins important for pairing of homologues, as well as the formation and repair of meiotic double-strand breaks (DSBs). In many organisms, the axes and associated proteins, which are known as “linear elements" (LinEs) in S. pombe, are held in alignment through formation of the synaptonemal complex (SC), and are sometimes considered to be the “lateral elements” of the SC. Here, Chuang and Smith use high-resolution microscopy, including structured illumination microscopy (SIM) to examine GFP- tagged strains of S. pombe. Specifically, they characterize the physical distribution of a number of LinE proteins as well as the cohesin subunit Rec8. In agreement with previous work, LinE proteins appear as dot-like structures during early and late prophase, but during the “horsetail stage” in which the nuclei undergo extensive telomere-driven motion, they appear as short linear fragments that do not extend along the full length of chromosomes, consistent with the idea that LinEs may be discontinuous. Recent studies from other groups have reported very similar observations; indeed I was unable to determine what aspects of this manuscript are novel, other than the finding that some LinE structures appear to be sensitive to hexanediol, a result I find questionable (see below). In my view, the transition from dot-like to linear structures likely reflects the highly elongated chromosome configuration present during the horsetail stage, rather than a functional difference in association of LinE proteins.

Reviewer 2 Comments for the Author:
I find the presentation of the work to focus inordinately on prior results and interpretations from the authors’ group while ignoring other relevant published data. In particular, I do not agree with the foundational statement in this manuscript that “LinE proteins are distantly related to the synaptonemal complex (SC) proteins of other species.” LinE proteins are related to chromosome axis proteins in other species, but this is a structure distinct from the SC central region. SCs have been found to be absent in S. pombe as well as a few other fungi and other cases, such as Drosophila spermatocytes. One LinE protein, Rec27, shows BLAST homology to a central region protein from C. elegans (SYP- 2), but this is almost certainly spurious, stemming from the fact that both are coiled-coil proteins, since an evolutionary relationship between these proteins is not supported by phylogenetic analysis.

We have added discussion of the recent publication by Ding et al. (Chromosoma 2021); see below. We asked the Editor and Reviewer 2 for references for other relevant research mentioned by Reviewer 2, underlined here and below, but we have not received any references. Without knowledge of the "other relevant published data," we cannot further revise our manuscript.

Perhaps more egregiously, the work fails to cite a recent paper from Ding et al. (https://doi.org/10.1007/s00412-021-00757-w), which included data that overlap extensively with the results presented here, as well as some contradictory findings. In particular, both this manuscript and the published paper also show that LinE proteins form both dot-like and linear structures, the latter being the dominant form during the horsetail stage. They also both demonstrate that the LinE proteins other than Rec10 are interdependent for their association with meiotic chromosomes, and that Rec10 is required “upstream” of all other LinE proteins. However, in contrast to the current manuscript, Ding et al. concluded that LinE structures are stable and not sensitive to disruption by hexanediol. Clearly, this paper must be cited and the differences in the observations should be directly addressed and ideally explained in a convincing way. It seems unlikely that strain differences or culture conditions would have such a marked effect on the hexanediol sensitivity of these structures. I find the results of Ding et al. somewhat more

convincing for 2 reasons: the conclusion that the LinE proteins are insensitive to hexanediol were corroborated by photobleaching experiments that also indicated that LinE proteins are stably bound (i.e., they do not show rapid recovery in FRAP analysis), and also because the hexanediol was added and washed out while imaging. Most cellular assemblies that are sensitive to hexanediol can rapidly reassemble upon removal of the solvent, so this should be tested for the LinE proteins. This is important because extended exposure to hexanediol likely kills cells, making it difficult to conclude that disruption of the LinE structures is biologically meaningful. [We do not say it is.]

We have added on page 13 discussion of Ding et al. (Chromosoma 2021), which was published just as we were submitting our paper. We think their results, like ours, indicate the LinEs are hexanediol- sensitive. For example, their Figure 5 shows altered structures or much less GFP intensity for Rec10- GFP (panels a, d, and f) and Mug20-GFP (panel c) after treatment with hexanediol than before. After washout of hexanediol, there is clear recovery of GFP intensity for Mug20-GFP (panel c) and Rec10- GFP (panel f). In Figure S4, both Rec25-GFP and Mug20-GFP show similar hexanediol-sensitivity.
Because no quantitative analyses of their images were published, it is difficult to make a direct comparison of their data with ours. Our quantitative data (Figure S11) show that the GFP intensity is quite significantly reduced ($p < 0.001$, < 0.01, < 0.01, and < 0.05 for the four LinEs) after hexanediol addition. Thus, both our data and many of Ding's data show hexanediol-sensitivity and, for some of Ding's data, recovery within about 5 min after hexanediol removal. We say on page 13, “Thus, the two groups interpreted similar results differently.” We think readers should examine and interpret the data as they think appropriate.

All tagged strains should be quantitatively validated by characterizing the duration of meiosis, the crossover distribution, and spore viability.

We and others have published data showing high activity of the GFP-tagged proteins used here. Please see Davis et al. (Curr Biol 2008) for Rec25-GFP and Rec27-GFP, and Fowler et al. (Mol Cell 2013) for Rec10-GFP, Mug20-GFP, and Rec8-GFP. On page 13 (line 430) of the previous submission, we cited these sources.

As far as I am aware, the Smith lab is the only group that considers S. pombe to show crossover interference. The formal definition of CO interference is that crossovers in two intervals on the same chromosome quantitatively “interfere” with each other – that is, the probability of observing a CO in a particular interval is lower if there there is a crossover in a nearby interval along the same chromosome. This has been tested in S. pombe and found not to be the case (https://doi.org/10.1093/genetics/137.3.701). The model previously proposed by Fowler et al. (2018) seems to equate DSB interference with crossover interference. I feel that the authors have cherry- picked results from previous papers that support equating DSB interference with CO interference while ignoring much more compelling evidence that these are separate phenomena – e.g., that CO interference still functions when DSB interference is disrupted, or if breaks are induced by irradiation or DNA-damaging agents. S. pombe also lacks synaptonemal complexes (SCs), and mounting evidence indicates that this structure is essential for crossover interference, although this remains controversial. Further evidence against CO interference is that the pathway that gives rise to COs in S. pombe is different from the ZMM pathway that is subject to interference in other organisms. The concept of CO interference is not necessarily related to the work presented here, but the framing of the work in this way seems misguided to me, and I believe most researchers in the field would share this view.

The URL above is for Munz (Genetics 1994), which is the only paper, to our knowledge, other than Fowler et al. (PNAS 2018) with data on *S. pombe* crossover interference. In Fowler et al., we used an assay for interference (scoring over a thousand double recombinants in 17 repeat experiments) more sensitive than the one Munz used (scoring only 0 – 25 double recombinants in each interval pair tested). Because his intervals were large (often ~20 cM or more), the low interference we found would have been nearly impossible to detect with such limited numbers of recombinants. Furthermore, Munz observed positive interference in two of the fifteen crosses reported ($I = 0.33$ and 0.29). In the interval pair we used (*ura2 - leu2 - lys7*), Munz observed 34 and 12 single crossovers and 1 double crossover among 373 random spores tested, giving $I = 0.09$, indicating slight positive interference, but not significant because of the low numbers. Thus, as we discussed in Fowler et al. (PNAS 2018), we see no contradictions in the data, and our conclusion that *S.*

pombe has crossover interference, though weak (I ≈ 0.26 from our extensive analyses), is consistent with both publications.

We discuss crossover interference because the LinE proteins investigated here form the clusters we propose are intimately involved in this nearly universal feature of meiosis. Fowler et al. proposed that crossover interference arises from DSB interference, both of which were assayed and found to be positive in wt but negative in *tel1Δ* mutants. We asked Reviewer 2 for references for crossover interference functioning in the absence of DSB interference, but we received no reply. Species differ, and the mechanisms in one species may or may not pertain to another species. Our proposed mechanism for crossover interference is based on data from *S. pombe* and may pertain to other species.

While some organisms have true DSB "hotspots," in others (e.g., Drosophila, C. elegans, Arabidopsis) there are merely "warm" and cooler regions. DSB maps have now been generated for considerably more organisms than cited here, including several mammals and plants.

On page 2, line 45, of the previous submission, we cited DSB hotspot patterns in *S. cerevisiae*, *S. pombe*, and mice; we have now added reference to a review by Nambiar et al. (2019), which discusses these three species and *A. thaliana*, the four species to our knowledge for which genome-wide DSB frequencies have been determined. We asked for additional references but received none.

The idea that the "dotty" LinE structures reflect clustering of potential sites to mediate DSB interference was presented in previous work from the group and is reiterated here as a model to explain/interpret the observations. However, I don't think that the data in this manuscript provide any support for this notion, particularly for the idea that the dots somehow represent a structure that mediates DSB interference. While the duration of linear LinE structures is modulated by the absence of DSBs, this likely reflects alterations in meiotic progression and the length of the horsetail stage rather than a direct consequence.

The LinE structures we report are at the heart of our clustering model, and it is appropriate to discuss the relation of our observations to the model.

I'm not sure what the authors mean by: "The topoisomerase-like protein Spo11, first identified in budding yeast, contains the active site for meiotic DSB formation in multiple experimental organisms" (lines 41-42). While strictly true, this is an awkward way to describe Spo11's role. Additionally, the correlation between binding of LinE proteins and DSB hotspots seems as likely to reflect a role for LinE proteins in repair of DSBs as it does a causal role in DSB formation. The manuscript also seems to ignore data linking transcriptional activity to DSB formation in S. pombe (https://doi.org/10.1534/genetics.116.197954).

We do not understand what is awkward with our description of Spo11's activity. Its role important for the work described here is formation of DSBs, which we say. It is closely related to topoisomerase VI- type enzymes, it contains a Tyr residue that makes a covalent link to DNA upon cleavage of the DNA, it was first identified *in S. cerevisiae* as a sporulation-deficient mutant (Esposito and Esposito, Genetics 1969) and found to be covalently linked to DNA during meiosis (Keeney et al., Cell 1997), and its homolog is found in many species.

It is not just the genome-wide correlation of LinE binding and DSB hotspots that is critical here. Rather, it is also that DSB formation is largely dependent on Rec25, Rec27, and Mug20 (Davis et al., Curr Biol 2008; Fowler et al., Mol Cell 2013; Ma et al., Nucleic Acids Res 2017) and entirely dependent on Rec10 (Cervantes et al., Mol Cell 2000; Ellermeier and Smith, PNAS 2005). In *S. pombe*, and likely other species, a given transcriptional activator acts at only a limited number of DSB hotspots; e.g., Atf1-Pcr1 activates the *ade6-M26* hotspot, but deletion of *pcr1* leaves most hotspots intact (Steiner and Smith, Mol Cell Biol 2005). In contrast, the small LinE proteins are required for most DSBs at nearly all hotspots (op cit).

We are aware of only one published datum suggesting that LinEs are involved in DSB repair. About half of the foci of Rad51 DSB repair protein co-localize with Rec10 foci (Lorenz et al., Chromosoma

2006). This could reflect Rec10 remaining bound to chromatin after a DSB is made and Rad51 then binding; but Rec10 may not have any role in the DSB repair. This is consistent with half of the Rad51 foci not being near Rec10 foci. We cited this study on page 15 (line 503) of the previous submission where we discuss the proposal, supported by our new data, that LinEs are involved in DSB repair, as well as in their established role in DSB formation.

I don't understand why the authors conclude that the LinE proteins form a complex in the cytoplasm. Rec25, Rec27, and Mug20 are all very small proteins (<20 kD) and would thus readily pass through nuclear pores without a requirement for an NLS. Also, why would LinE structures differ in zygotic vs. azygotic meiosis?

In Rec10-NLS mutants, the small LinE proteins form a complex with Rec10 in the cytoplasm (Wintrebert et al., J Cell Sci 2021). We think it simplest to suppose that in wt they form a complex in the cytoplasm and then are carried into the nucleus by Rec10, but we've stated that other scenarios are possible.

In zygotic meiosis, the homologs are in the same cell for the first time, whereas in azygotic meiosis they have been in the same cell for generations and have had the opportunity to coalesce at least partially before meiosis begins. Previous reports (e.g., Bahler et al., Curr Genet 1991; Ding et al., Chromosoma 2016; Escorcia et al., Open Biol 2021) also indicate differences in chromosome behavior in the two types of meiosis.

Ultimately, I find this work to be of insufficient novely or impact for publication in its current form. The discrepancies between these findings and the published results of Ding et al. (2021 Chromosoma) should be addressed before this manuscript is reconsidered.

In the revision we have discussed the differences in interpretation of our data and those of Ding et al. (Chromosoma 2021), described above. We think our work reported here, by comparing meiotic chromosome behavior under a variety of conditions, explains seeming discrepancies in the literature. This knowledge is essential to productively continue studying meiosis in *S. pombe*, which has many features making it one of the best model organisms for elucidating the molecular mechanisms of meiotic recombination and its control.

Reviewer 3 Advance Summary and Potential Significance to Field:
THis is **a detailed study** from the Smith lab **using live-cell microscopy** for an **exhaustive re-examination** of the localization of element proteins in fission yeast and their inter-dependence. S. pombe has typically been reported not to have a synaptonemal complex; this study uses microscopy to argue that there are indeed linear elements that appear to have **structures similar to an SC, agreeing with recently published work**. The authors also use mutations in different components to perturb the structures and determine inter-dependence. Finally, they compare a normal zygotic meiosis to a manipulated azygotic meiosis (using pat1-ts, which has been heavily used in the field in earlier days) to compare the behavior of the proteins under these different conditions. The use of pat1ts can impose artifacts on meiosis so **it is helpful to have a direct comparison**.

Reviewer 3 Comments for the Author:
This is a descriptive study that relies entirely on microscopic observations, that is of particular interest to the afficionado**. It confirms and also expands prior observations from more technically-challenged times.**

Concerns are generally minor.
1) Although the abstract states "Here, we investigated LinE configurations and 15 [sic] distributions in live cells using super-resolution fluorescence microscopy" only one figure in the paper appears to use SIM, although it is prominently featured in the supplemental. Authors should ensure that each figure indicates the modality employed for imaging. Fig S8 should be moved to the main figures.

We stated in the legends to each figure the type of microscope used. Figures 1, 2, 6, S1, S2, S4, S5, S6, S8, S12, and S13 used SIM, and Figures 5, S3, S9, and S10 used DeltaVision; i.e., most of the data in the main text used SIM. We think Figure S8, showing an example of the computer-aided analysis of images, pertains to methods and is properly in the Supplemental Information.

2) Rec8 is reported to be resistant to Hexane-diol but there certainly appears to be a reduction in signal lin the Rec10GFP-Hex sample (Fig 5, col 2 row 2). It is not clear that this figure makes an essential contribution, and if it is kept, it should include the quantitation in S11. The additional related data in S10 is also poorly labeled and described. Are these the same cells imaged at 0 and 5'? Or different cells? What's the conclusion? It appears presented because it correlates with observations in other species.

Rec8-GFP is quantified in Figure S11, which is based on nine cells and shows no significant reduction of GFP intensity upon addition of hexanediol. This is our control to emphasize that the highly significant reductions of LinE-GFP intensity ($p < 0.01$ in three cases; $p < 0.05$ in the fourth) means sensitivity to hexanediol. In Figure 5, we have replaced the image for Rec8-mCherry in the Rec10- GFP cell with a more representative figure, which clearly shows hexanediol-resistance, as shown by the other images in Figure 5 and quantified in Figure S11.

We have reformatted Figure S10 and rewritten its legend, as noted in comments to Reviewer 1.

3) The authors should leave the azygotic pat1 meiosis data (e.g., Fig 2) in the supplemental and show that data on zygotic meiosis (e.g., Fig S9). Jumping from one to the other is disorienting to the reader. They can explain that the highly quantitative data are derived from the azygotic.

We consider this a matter of style. We prefer to present a new observation using improved methods and then show our data using the previous methods for direct comparison. This clarifies why in some cases our new conclusions differ from previous conclusions. The alternative would be to present all of our new data, and then go through the same types of experiments with the previous methods. We think the reader would then have to jump back to the first part of the paper to understand the significance of the repeats using previous methods.

4) The authors are missing two very relevant recent citations, and should discuss how these fit with their observations and model.

•Ding et al Chromosoma. 2021 Apr 7. doi: 10.1007/s00412-021-00757-w.
Linear elements are stable structures along the chromosome axis in fission yeast meiosis

This paper was published just as we were submitting our manuscript to *J Cell Sci*. We wished to proceed with our submission while recognizing that we would address the paper by Ding et al. in our revision. This we have done (see Discussion on page 13).

•Escorcia et al Open Biol. 2021 Feb;11(2):200357. doi: 10.1098/rsob.200357. Epub 2021 Feb 24.
A visual atlas of meiotic protein dynamics in living fission yeast

This paper deals with only one protein, Rec27-GFP, that we deal with but adds little to our observations. We have now noted this paper and its observations relevant to our data on pages 4 and 15.

First decision letter (10 August 2021)

MS ID#: JOCES/2021/259061
MS TITLE: Dynamic configurations of meiotic DNA-break hotspot determinants
AUTHORS: Yu-Chien Chuang and Gerald R Smith
ARTICLE TYPE: Research Article

Dear Dr. Smith,

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to: https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area. (Corresponding author only has access to reviews.)

As you will see, the reviewers raise a number of substantial criticisms that prevent me from accepting the paper at this stage. They suggest, however, that a revised version might prove acceptable, if you can address their concerns. If you think that you can deal satisfactorily with the criticisms on revision, I would be pleased to see a revised manuscript. We would then return it to the reviewers.

We are aware that you may be experiencing disruption to the normal running of your lab that makes experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

Please ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion.

I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. Please attend to all of the reviewers' comments. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

Reviewer 1

Advance summary and potential significance to field

I am satisfied with the reply and the changes made by the authors.

Comments for the author

N/A

Reviewer 2

Advance summary and potential significance to field

S. pombe does not form typical synaptonemal complex. However, prior studies have provided evidence for a specialized meiotic chromosome structure, composed of LinE proteins. It is possible that this structure is related to the meiotic chromosome axis/ lateral elements of the SC in other organisms, although it appears to lack the transverse filaments that connect homologous chromosomes. This data presented here is significant because studying this structure could provide insights into how the chromosomes axis regulates meiotic recombination. The limitation of this work is that it is almost entirely descriptive. In my opinion this does not preclude publication, but the writing and presentation must reflect this. This paper is far too speculative considering the data it presents. In addition, the organization, particularly with the Figures, makes this paper harder to follow than it needs to be. It would help if the authors would be more responsive to some of the changes suggested in the previous reviews.

Comments for the author

1) It is interesting that assembly of longer linear structures only occurs at 25deg C. Unfortunately, there is no data in this paper to indicate if there are consequences to this. Can the authors comment on the how the temperature affects crossing over or DSB formation?

2) There is a large number of supplemental figures and some elements could be in the main Figures. Efforts should be made to reduce the number of supp figures and put quantitation and images in the same figure. Some examples include: it would be useful to have the key parts of Fig S3 in Fig 1 to compare to 1E, the quantitation in Figure S11 should be in Figure 5, Figure 2 and 3 could be the same figure, Fig 4 quantitation should be with the images. It appears similar comments were made in the previous reviews but they have not been adequately addressed.

3) Line 233: can the author make it clear at the end of this paragraph that linear structures did not appear at 34 deg.

4) The authors make a significant point out of the transition from dotty to long linear structures and back again. Indeed, SC does this too. Like SC, the dotty stage could be sites where the axis first forms, which is followed be more assembly of axis until a peak is achieved at which point the structure starts to dissolve and the dots appear again. There is no evidence presented to indicate that the return to a dotty structure has any function except to represent the disassembly of the meiotic axis which normally occurs before metaphase I. The authors should reduce the speculation on this observation.
It is pretty striking that some of these axis elements can retained into MI in some mutants. Has this been confirmed with markers for MI, like observing LinE proteins and a meiotic spindle at the same time?

5) Line 401: the authors comment on the “nearly continuous” chromosomal structures. With the high-resolution analysis presented here, can the authors conclude they are continuous? Is there evidence for one continuous structure per chromosome? Can the lengths be measured?

6) Line 408: it is misleading to conclude that S. pombe has crossover interference. Interference is a quantitative measurement, and while S. pombe may show some measurable level of interference with large sample sizes, this should be not equated to what organisms with SC do. Line 414 is also misleading. It is reasonable to suggest the S. pombe axis proteins form structures similar to the axis of SC containing organisms, but there is no evidence to suggest S. pombe has functional equivalent of transverse filaments. Previous reviewers commented on these two over interpretations of the data and the authors need to be more responsive.

7) Line 427: I don’t understand the sentence that two groups interpreted similar results differently. This is not an interpretation. Either the treatment reduced line-GFP intensity or it did not. Perhaps the authors need to write that the previous results were not quantified the same way.

8) Line 516-520: I don’t understand how the authors conclude that LinE proteins complexes bind DSB hotspots. The author should use caution when making links between LinEs and DSBs because they assemble in the absence of DSBs.

9) There is no evidence for much of the model in Figure 7 (and statements in the Discussion and line 73 of the Introduction). Most of panel B is pure fantasy and not based on any data in the paper (specifically the relationship between LinE protein localization, DSB sites, and their clustering). The entire Figure should be deleted or only show that this paper has found (the structure of the chromosome axis, e.g continuous or not).
Similarly, the title is misleading and should be changed to a more conservative tone.

Reviewer 3

Advance summary and potential significance to field

This is a detailed description of the events associated with linear element proteins (LinEs) and their dynamics during meiosis in fission yeast. It is related to a recently published paper from another lab (Ding et al 2021) with generally similar findings. This examination is based entirely on imaging, with mechanistic details only inferred. It is of interest to the afficionado.

Comments for the author

The authors have generally dealt with my concerns, although I still consider the inclusion of azygotic meiosis at 34°C in the primary body of the manuscript to be a distraction.

Reviewer 4

Advance summary and potential significance to field

The authors have characterized the localization of several "LinE" (Linear Element" proteins over the course of meiotic prophase. In agreement with other published work, they find that these proteins form dotty, apparently discontinuous structures along meiotic chromosomes, become linear stretches as the chromosomes elongate during the "horsetail stage," when nuclei are actively pulled by their telomeres though the cytoplasm, and become dotty again in late prophase; the latter transition is perturbed by mutations that affect meiotic recombination. They also report that the localization of some LinE proteins to the chromosomes is partially disrupted by treatment of cells with 1,6-hexanediol, a solvent that is thought to disrupt hydrophobic interactions within cells. They interpret these findings in light of prior evidence that LinE proteins promote break induction during meiosis, and they argue that the LinE proteins are more similar than previously realized to synaptonemal complexes in other organisms.

Comments for the author

The authors have chosen to rebut rather than incorporate most of my previous comments on their original submission, other than citing and mentioning other recent work that overlaps extensively with their current findings. I can understand this response, since my review was quite critical and my comments were not expressed in the most kindly fashion, but unfortunately this means that most of the issues that I found problematic in the original submission remain in the current revision. The evidence presented here is fine as far as it goes, but most of the functional interpretations are purely speculative and are not tested by the experiments or directly supported by the data. In particular, I do not find that the results presented here shed light on the mechanism or regulation of DSB formation or repair, nor do they "[force] re-evaluating the relation of LinEs and the synaptonemal complex in other species." Thus, I think the presentation should be re-revised prior to publication.

The LinE is structurally and functionally analogous to the meiotic chromosome axis in other organisms, and includes homologues of axis proteins from other species, including Red1 and Hop1 from S. cerevisiae. In all species, axes are essential meiotic regulatory platforms that control the formation and repair of breaks, and thus play essential roles in homologue pairing. Axis proteins also play widely conserved roles in meiotic checkpoints. The data presented here are worth documenting but they do not shed much light on the functions of LinE proteins. In particular, the findings do not suggest that LinE proteins do anything that axis proteins do not do in other species, or that the LinEs resemble SCs. The claim that "Rec27 and Mug20 have amino-acid sequence similarities to C. elegans SYP-2 (an SC protein) and DDL-1 (a protein associated with the SC), respectively (Fowler et al., 2013)" is, in my view, misleading. Rec27 has a short region of potential coiled-coil formation, like SC central region proteins in other species, but some axis proteins, namely SYCP2/3, do as well. SYP-2 is a SC protein in C. elegans that does not have obvious homologues outside of Caenorhabditis (see https://doi.org/10.1101/2021.06.16.448737), let alone in fission yeast. DDL-1 is not an SC protein per se, in the sense that it is not required for SC assembly, nor does it seem to be broadly conserved. If the authors wish to argue that Rec27 and Mug20 are homologues of SC proteins they should present multiple sequence alignments and best reciprocal BLAST data; here they only cite previous work, which found only short regions of similarity, which my own analysis has indicated are likely false positives. As far as I can judge, the LinE proteins do not resemble SC proteins, which are typically much larger and are mostly comprised of predicted coiled-coils.

The evidence presented here that 1,6-hexanediol reduces the localization of some LinE proteins suggests that their association with the axis depends to some extent on hydrophobic interactions. However, this does not imply that they have liquid-like properties or are functionally related to

SCs. This data in particular has been seriously overinterpreted, particularly in light of the evidence from Ding et al., that these proteins do not show fluid-like dynamics along the axis. The functional significance of the previous SC findings, as I understand it, is that the SC may facilitate the movement of recombination proteins via 1-dimensional diffusion along paired chromosomes, so evidence should be presented here for similar diffusion if a functional analogy is to be drawn. I do not find that the data justify the statement (lines 266-267): The linear LinE structures implied that S. pombe forms structures functionally similar to the SC in zygotic meiosis at 25°C when examined in live cells."

I have no objection to the authors' speculation on these topics, but I feel that speculation and data presentation are interwoven too tightly in this presentation. I think the title should be changed because LinE proteins are not only "hotspot determinants," but play a key role in structuring meiotic chromosomes. I think that the results should be presented in a factual, unembellished fashion and that speculation should be clearly framed as such and left to the Discussion.

First revision

Cover letter (7 September 2021)

Dear Dr. Glover,

Yu-Chien Chuang and I have revised our manuscript "**Dynamic configurations of meiotic DNA-break hotspot determinants**" (MS ID#: JOCES/2021/258770) and resubmitted it to the Journal of Cell Science. We greatly appreciate your reconsidering our manuscript for publication.

As before, Reviewers 1 and 3 were quite positive about the substance and value of our research. As suggested by Reviewer 3, we have explained that we include data for meiosis at 34°C for comparison with much previous literature at this temperature. We have responded to the points raised by Reviewers 2 and 4 (see "Response to Reviews") and made several of the changes they suggest. We have rearranged the figures as suggested by Reviewer 2 and in accord with the policy of the journal. Other changes are noted in red on a copy of the manuscript we uploaded.

We greatly appreciate your help in making our research available to the community through publication in the Journal of Cell Science.

Yours sincerely,

Gerald R. Smith
Professor, Division of Basic Sciences

Author response to reviewers' comments

Below, we have **underlined and bolded the many positive comments** by the reviewers, for which we are grateful. Our responses to other points are in red font and enclosed in square brackets when placed within a reviewer's paragraph.

Reviewer 1 Advance Summary and Potential Significance to Field:
I am satisfied with the reply and the changes made by the authors.

Reviewer 1 Comments for the Author:
N/A

Reviewer 2 Advance Summary and Potential Significance to Field:
S. pombe does not form typical synaptonemal complex. However, prior studies have provided evidence for a specialized meiotic chromosome structure, composed of LinE proteins. It is possible

that this structure is related to the meiotic chromosome axis/ lateral elements of the SC in other organisms, although it appears to lack the transverse filaments that connect homologous chromosomes. **This data presented here is significant because studying this structure could provide insights into how the chromosomes axis regulates meiotic recombination.** The limitation of this work is that it is almost entirely descriptive. In my opinion this does not preclude publication, but the writing and presentation must reflect this. This paper is far too speculative considering the data it presents. In addition, the organization, particularly with the Figures, makes this paper harder to follow than it needs to be. It would help if the authors would be more responsive to some of the changes suggested in the previous reviews.

Reviewer 2 Comments for the Author:
1. It is interesting that assembly of longer linear structures only occurs at 25deg C. Unfortunately, there is no data in this paper to indicate if there are consequences to this. Can the authors comment on the how the temperature affects crossing over or DSB formation?

Hyppa et al. (2014) compared the genome-wide DSB frequencies at 25˚C and 34˚C. The DSB pattern is similar at both temperatures, but they reported a few DSB hotspots specific for 25˚C or 34˚C. We have not tested crossing over at 34˚C. Li and Smith (1997) showed that intragenic recombination at *ade6* is reduced to half at 34˚C compared to 25˚C. The dramatic difference in LinE structures at 25˚C and 34˚C (Figs. 1A and 1B; Figs 2B and 2C) suggests that features of meiotic recombination yet to be discovered depend on the altered LinE structures.

2. There is a large number of supplemental figures and some elements could be in the main Figures. Efforts should be made to reduce the number of supp figures and put quantitation and images in the same figure. Some examples include: it would be useful to have the key parts of Fig S3 in Fig 1 to compare to 1E, the quantitation in Figure S11 should be in Figure 5, Figure 2 and 3 could be the same figure, Fig 4 quantitation should be with the images. It appears similar comments were made in the previous reviews but they have not been adequately addressed.

We appreciate the reviewer's suggestion and have rearranged the sequence of our figures and moved some of the information in the supplemental figures to main-text figures.

3. Line 233: can the author make it clear at the end of this paragraph that linear structures did not appear at 34 deg.

We appreciate the reviewer's suggestion and have added this point in lines 244 – 245.

4. The authors make a significant point out of the transition from dotty to long linear structures and back again. Indeed, SC does this too. Like SC, the dotty stage could be sites where the axis first forms, which is followed be more assembly of axis until a peak is achieved at which point the structure starts to dissolve and the dots appear again. There is no evidence presented to indicate that the return to a dotty structure has any function except to represent the disassembly of the meiotic axis which normally occurs before metaphase I. The authors should reduce the speculation on this observation.

The persistence of LinE dotty forms after MI in DSB-deficient strains (Figs. 3C and 3F) strongly suggests that the LinE structures must remain until DSBs have formed. We discuss this new insight on lines 517 – 518.

It is pretty striking that some of these axis elements can retained into MI in some mutants. Has this been confirmed with markers for MI, like observing LinE proteins and a meiotic spindle at the same time?

We did not visualize the meiotic spindle in our experiments; instead, we determine meiotic stages by observing nuclear division using Hoechst 33342 staining, which is often used in live, meiotic microscopy studies in *S. pombe* (*e.g.,* Escorcia et al. 2021). We noted above the importance of retention of LinE structures after MI.

5. Line 401: the authors comment on the "nearly continuous" chromosomal structures. With the high-resolution analysis presented here, can the authors conclude they are continuous? Is there

evidence for one continuous structure per chromosome? Can the lengths be measured?

We did not conclude LinE proteins form a single continuous structure across a chromosome. As Figures 1A and S1A show, we found, however, that these structures are longer than previously observed except by Ding et al. (2021), as we note on pages 7, but there may be gaps in linear structures. The reviewer may have overlooked that we quantified the length of linear structures of all four LinE proteins (Fig. 7B and Tables S3 and S4).

6. Line 408: it is misleading to conclude that S. pombe has crossover interference. Interference is a quantitative measurement, and while S. pombe may show some measurable level of interference with large sample sizes, this should be not equated to what organisms with SC do. Line 414 is also misleading. It is reasonable to suggest the S. pombe axis proteins form structures similar to the axis of SC containing organisms, but there is no evidence to suggest S. pombe has functional equivalent of transverse filaments. Previous reviewers commented on these two over interpretations of the data and the authors need to be more responsive.

Fowler et al. (2018) showed there is positive meiotic crossover interference in *S. pombe* meiosis with standard genetic assays. We think it is appropriate to cite this work and to discuss their findings with other studies of crossover interference.

We suggest *S. pombe* LinE proteins form a structure that shares some structural and functional features of the SC based on the following factors: they are meiosis-specific proteins sharing limited homology with SC proteins of other species; they form linear structures along the chromosome; they are required for meiotic recombination; and they are sensitive to 1,6-hexanediol (HD). We do not state the *S. pombe* has an SC.

7. Line 427: I don't understand the sentence that two groups interpreted similar results differently. This is not an interpretation. Either the treatment reduced line-GFP intensity or it did not. Perhaps the authors need to write that the previous results were not quantified the same way.

We appreciate the reviewer's suggestion, and we have noted it in our paper (lines 428 – 439). Our quantification shows that HD reduces LinE-GFP intensity (Fig 6C). Ding et al. did not report quantification, but to our eyes the intensity goes down upon HD addition and goes up again upon HD restoration. Ding et al. said that LinE structures are not HD- sensitive, but our interpretation of their figures differs.

8. Line 516-520: I don't understand how the authors conclude that LinE proteins complexes bind DSB hotspots. The author should use caution when making links between LinEs and DSBs because they assemble in the absence of DSBs.

Fowler et al. (2013) showed LinE proteins bind to meiotic DSB hotspots with high specificity, and their density (amount of bound protein/kb of DNA) is often 20 – 80 times higher at DSB hotspots than the genome median. In the absence of LinEs, DSBs are strongly reduced at most hotspots. Thus, these proteins are DSB hotspot determinants. They bind the hotspots independent of DSB formation and thus likely before DSBs are made.

9. There is no evidence for much of the model in Figure 7 (and statements in the Discussion and line 73 of the Introduction). Most of panel B is pure fantasy and not based on any data in the paper (specifically the relationship between LinE protein localization, DSB sites, and their clustering). The entire Figure should be deleted or only show that this paper has found (the structure of the chromosome axis, e.g continuous or not).

Figure 7 is a model. We think scientific models should incorporate previously reported findings as well as the current findings and that useful models also propose features to motivate further experiments and discoveries.

Wintrebert et al. (2020) showed that the LinEs enter the nucleus using the NLS of Rec10. Estreicher et al. (2012) showed Rec10 and Mug20 co-localize by microscopy. Fowler et al. (2013) showed that LinE complexes bind meiotic DSB hotspots by genome-wide analysis and that Rec25 and Mug20 co-localize by microscopy. Fowler et al. (2018) showed LinE complexes form clusters of DSB hotspots

within 200 kb regions. These published data strongly support the model we draw.

Our findings add more information supporting the previously published clustering model of DSB and crossover interference.

Similarly, the title is misleading and should be changed to a more conservative tone.

Our paper studies the dynamic configurations of LinE proteins, and LinE proteins are meiotic DNA-break hotspot determinants (Fowler et al. 2013), as the title says.

Reviewer 3 Advance Summary and Potential Significance to Field:
This is a detailed description of the events associated with linear element proteins (LinEs) and their dynamics during meiosis in fission yeast. It is related to a recently published paper from another lab (Ding et al 2021) with generally similar findings. This examination is based entirely on imaging, with mechanistic details only inferred. It is of interest to the afficionado.

Reviewer 3 Comments for the Author:
The authors have generally dealt with my concerns, although I still consider the inclusion of azygotic meiosis at 34°C in the primary body of the manuscript to be a distraction.

We include data at 34°C because so much research on *S. pombe* meiosis has used this condition, and we wish to compare it directly with results at 25°C, which is also widely used.

Reviewer 4 Advance Summary and Potential Significance to Field:
The authors have characterized the localization of several "LinE" (Linear Element" proteins over the course of meiotic prophase. In agreement with other published work, they find that these proteins form dotty, apparently discontinuous structures along meiotic chromosomes, become linear stretches as the chromosomes elongate during the "horsetail stage," when nuclei are actively pulled by their telomeres though the cytoplasm, and become dotty again in late prophase; the latter transition is perturbed by mutations that affect meiotic recombination. They also report that the localization of some LinE proteins to the chromosomes is partially disrupted by treatment of cells with 1,6-hexanediol, a solvent that is thought to disrupt hydrophobic interactions within cells. They interpret these findings in light of prior evidence that LinE proteins promote break induction during meiosis, and they argue that the LinE proteins are more similar than previously realized to synaptonemal complexes in other organisms.

Reviewer 4 Comments for the Author:
The authors have chosen to rebut rather than incorporate most of my previous comments on their original submission, other than citing and mentioning other recent work that overlaps extensively with their current findings.I can understand this response, since my review was quite critical and my comments were not expressed in the most kindly fashion, but unfortunately this means that most of the issues that I found problematic in the original submission remain in the current revision. [We were not provided the references we requested from this reviewer and thus could not respond to many comments.] The evidence presented here is fine as far as it goes, but most of the functional interpretations are purely speculative and are not tested by the experiments or directly supported by the data. In particular, I do not find that the results presented here shed light on the mechanism or regulation of DSB formation or repair, nor do they "[force] re-evaluating the relation of LinEs and the synaptonemal complex in other species." Thus, I think the presentation should be re-revised prior to publication.

The LinE is structurally and functionally analogous to the meiotic chromosome axis in other organisms, and includes homologues of axis proteins from other species, including Red1 and Hop1 from S. cerevisiae. In all species, axes are essential meiotic regulatory platforms that control the formation and repair of breaks, and thus play essential roles in homologue pairing. Axis proteins also play widely conserved roles in meiotic checkpoints. The data presented here are worth documenting but they do not shed much light on the functions of LinE proteins. In particular, the findings do not suggest that LinE proteins do anything that axis proteins do not do in other species, or that the LinEs resemble SCs.

Published results and ours indicate that LinE proteins of *S. pombe* form a structure sharing many functional aspects of the SC based on the following factors: they are meiosis-specific proteins; they form linear structures along the chromosome; they are required for meiotic recombination; and they are sensitive to 1,6-hexanediol. Our current paper is on the dynamic structure of the LinEs and their dependence on proteins, DSB formation, and time during meiosis, and the effect of mutations in LinE proteins and others required for meiotic recombination. We relate these structural findings to previously reported LinE functions, such as DSB formation, DSB interference, and recombination.

The claim that "Rec27 and Mug20 have amino-acid sequence similarities to C. elegans SYP-2 (an SC protein) and DDL-1 (a protein associated with the SC), respectively (Fowler et al., 2013)" is, in my view, misleading. Rec27 has a short region of potential coiled-coil formation, like SC central region proteins in other species, but some axis proteins, namely SYCP2/3, do as well. SYP-2 is a SC protein in C. elegans that does not have obvious homologues outside of Caenorhabditis (see https://doi.org/10.1101/2021.06.16.448737), let alone in fission yeast. DDL-1 is not an SC protein per se, in the sense that it is not required for SC assembly [We are unaware of a reference for this statement.], nor does it seem to be broadly conserved. If the authors wish to argue that Rec27 and Mug20 are homologues of SC proteins they should present multiple sequence alignments and best reciprocal BLAST data [This was shown by previous studies; please see below.]; here they only cite previous work, which found only short regions of similarity, which my own analysis has indicated are likely false positives [We are unaware of a reference for this statement.]. As far as I can judge, the LinE proteins do not resemble SC proteins, which are typically much larger and are mostly comprised of predicted coiled-coils

Lorenz et al. (2004) showed that *S. pombe* Rec10 is a homolog of *S. cerevisiae* Red1. Fowler et al. (2013) and Ma et al. (2017) showed amino acid similarity between *S. pombe* Rec27 and *C elegans* SYP-2, and between *S. pombe* Mug20 and *C elegans* DDL-1.

The unpublished paper (URL above) states, "The SC is in all eukaryotic clades, and is essential in meiosis." *S. pombe* is a eukaryote that undergoes meiosis. The paper claims SC proteins have 1) high amino acid sequence diversity, 2) conserved coiled-coil domains, and 3) low coefficient of variation of protein length. But they mostly compared *Caenorhabditis* species, and it is not surprising that *S. pombe* has different sizes of coiled-coil domain proteins. A variety of protein structure prediction tools, such as Alphafold and Phyre2, indicate that all four LinEs form coiled-coils, like many SC proteins.

The evidence presented here that 1,6-hexanediol reduces the localization of some LinE proteins suggests that their association with the axis depends to some extent on hydrophobic interactions. However, this does not imply that they have liquid-like properties or are functionally related to SCs. This data in particular has been seriously overinterpreted, particularly in light of the evidence from Ding et al., that these proteins do not show fluid-like dynamics along the axis [See above for comparison of our data with those of Ding et al.]. The functional significance of the previous SC findings, as I understand it, is that the SC may facilitate the movement of recombination proteins via 1-dimensional diffusion along paired chromosomes, so evidence should be presented here for similar diffusion if a functional analogy is to be drawn. I do not find that the data justify the statement (lines 266-267): The linear LinE structures implied that S. pombe forms structures functionally similar to the SC in zygotic meiosis at 25°C when examined in live cells."

Rog et al. (2017) used hexanediol-sensitivity as one measure of liquid-like properties of SC proteins. We followed their reasoning. In addition, we have cited previous genetic studies and done many image analyses to support the conclusion that LinE proteins are functionally related to the SC.

As we noted above, the results of Ding et al (2021), like ours, show the LinEs are hexanediol-sensitive. We believe the two groups have different interpretations of 1,6- hexanediol data, as we discuss on page 13. See also response to Reviewer 2.

I have no objection to the authors' speculation on these topics, but I feel that speculation and data presentation are interwoven too tightly in this presentation. I think the title should be changed because LinE proteins are not only "hotspot determinants," [Fowler et al., 2013, showed they are

DSB hotspot determinants.] but play a key role in structuring meiotic chromosomes [This is a major point of our paper and is implied in the title.]. I think that the results should be presented in a factual, unembellished fashion and that speculation should be clearly framed as such and left to the Discussion. [Figure 7, in the Discussion, contains a model, clearly stated as such, encompassing previously reported and current facts and proposing features for future research.]

Second decision letter (5 October 2021)

MS ID#: JOCES/2021/259061
MS TITLE: Dynamic configurations of meiotic DNA-break hotspot determinants
AUTHORS: Yu-Chien Chuang and Gerald R Smith
ARTICLE TYPE: Research Article

Dear Dr. Smith,

We have now reached a decision on the above manuscript.

To see the reviewers' reports and a copy of this decision letter, please go to:

https://submit-jcs.biologists.org and click on the 'Manuscripts with Decisions' queue in the Author Area.

(Corresponding author only has access)

As you will see from their reports, the reviewers raise a number of substantial criticisms that prevent me from accepting your paper for publication.

I am very sorry to give you such disappointing news, but we are currently under great pressure for space and it takes a very enthusiastic recommendation by the referees for a manuscript to be accepted.

I do hope you find the comments of the reviewers helpful in allowing you to revise the manuscript for submission elsewhere, and many thanks for sending your work to Journal of Cell Science.

Reviewer 1

Advance summary and potential significance to field

This is a re-revision of a manuscript, which was previously evaluated possitively

Comments for the author

All my concerns were satisfactorily amended.

Reviewer 2

Advance summary and potential significance to field

I have not further suggestions. It is disappointing that the authors have chosen to rebut so many comments rather than accept the advice of multiple reviewers. The most contentious issues are not the data. Speculation is good and proposing new directions is good. But the title should reflect what is in the paper. Speculation need to be bound but what is known. They do not mention that, if interference exists in pombe, is quantitatively weak. I realize that the authors do not state pombe has SC, but they do say "infer this SC-like structure in S. pombe", which is close and

misleading. The fact that LinE proteins are required for hotspots does not make them hotspot determinants. And I still find it hard to understand the basis for some of the structural musing in the model. I leave it up to the editors to make the final judgement.

Comments for the author

see above

Reviewer 3

Advance summary and potential significance to field

This is a detailed description of the events associated with linear element proteins (LinEs) and their dynamics during meiosis in fission yeast. It is related to a recently published paper from another lab (Ding et al 2021) with generally similar findings. This examination is based entirely on imaging, with mechanistic details only inferred. It is of interest to the afficionado.

Comments for the author

My own comments were largely addressed previously.

The substantial concerns from other reviewers about speculative conclusions, however, have become an issue to me. I am troubled that authors continue to argue their way around those concerns, but really they do need to base conclusions on what they have demonstrated and not what they imagine to be true.

The fact that they essentially made no changes in the manuscript to deal with the legitimate concerns from two other reviewers, and simply argue their way around it in the response, seems to show a lack of good faith and an effort to bludgeon the reviewers and editor into acquiescence.

Author rebuttal letter (7 October 2021)

Dear Dr. Glover,

We have received the third set of reviews of our manuscript "**Dynamic configurations of meiotic DNA-break hotspot determinants**" (MS ID#: JOCES/2021/258770) but have found these reviews frustrating for their misunderstandings and lack of clarity. We find the reviewers' comments unfair and dismissive without stated reasons. We would like to have your own evaluation of our research report.

Several points made by the reviewers without justification include the following.
1. Rev. 2 says, "But the title should reflect what is in the paper." It does. Our data here show that the LinE proteins change their configuration during meiosis and therefore justify "Dynamic configurations." Published work (Fowler et al, *Mol Cell* 2013) shows that the *S. pombe LinE* proteins are required nearly genome-wide for DSBs at meiotic hotspots and bind to DSB hotspots with high specificity (a point apparently missed by Rev. 2) and therefore justify "meiotic DNA-break hotspot determinants."

2. Rev. 2 says, "They do not mention that, if interference exists in pombe, is [sic] quantitatively weak." *S. pombe* has highly significant crossover interference (I = 0.26 ± 0.05; 0.24 ± 0.07; 0.31 ± 0.04 in three separate sets of experiments; Fowler et al, *PNAS* 2018, Tables 1 and S2). These data justify our discussing interference and showing a model for it in our paper. If you wish, we will quote these interference data in our paper.

3. Rev. 3 says, "This examination is based entirely on imaging, with mechanistic details only inferred." That is not correct. Mechanistic details are based on many previously published data that we cite (for example, Fowler et al, op cit; Davis et al, *Curr Biol* 2008; Estreicher et al, *Curr Genet* 2012; Ma et al, *Sci Reports* 2017; Wintrebert et al, *J Cell Sci* 2021; Ding et al, *Chromosoma* 2021). Surely it is proper to use published data in discussing one's results.
4. Rev. 3 says, "… but they really do need to base conclusions on what they have demonstrated and not what they imagine to be true." Rev. 2 says, "Speculation need to be bound but what is known [sic]." Our conclusions are based on our data and previously published data. Our model (Figure 8, "Model for dotty to linear to dotty configurations of LinE structures") uses these data to propose how DSB formation and repair are controlled during meiosis. We clearly label it as a model and distinguish it from our conclusions. As we said in our response to the second reviews, "We think scientific models should incorporate previously reported findings as well as the current findings and that useful models also propose features to motivate further experiments and discoveries." Paul Nurse has amplified this view in his subsequent article "Biology must generate ideas as well as data" in *Nature* (https://www.nature.com/articles/d41586-021-02480-z). We hope you agree with this philosophy.

We would greatly appreciate your reconsidering, independently of the reviewers' points, our paper for publication. We will take into account your points to rewrite any part of the paper you think necessary. Thank you for your consideration.

Yours sincerely,
Gerald R. Smith
Professor, Division of Basic Sciences

Author rebuttal letter (19 October 2021)

Dear Dr. Way,

I am appealing to you, as Editor-in-Chief of the *Journal of Cell Science*, the decision on our revised manuscript "Dynamic configurations of meiotic DNA-break hotspot determinants" that Yu-Chien Chuang and I submitted to *J Cell Sci*. We think the reviewers' comments do not accurately and clearly reflect our paper and our response to the previous reviews. Attached is my letter appealing to Dr. Glover, the editor of our paper, explaining our views. I have not heard from Dr. Glover, and thus I am appealing to you to consider our paper, which we think should be published in the *Journal of Cell Science*.

I would greatly appreciate your reading the attached letter to Dr. Glover and the third set of reviews. For completeness, I have also attached the second set of reviews with our responses, our revised manuscript taking into account the first and second sets of reviews, and my letter of submission with that revised manuscript. If you wish the earlier versions of our manuscript, the reviews, and our responses, I will be glad to send them to you.

Thank you for considering our request.

Yours sincerely,

Gerry Smith

Gerald R. Smith
Professor, Division of Basic Sciences
Fred Hutchinson Cancer Research Center

Rebuttal response letter (1 November 2021)

MS ID#: JOCES/2021/259061
MS TITLE: Dynamic configurations of meiotic DNA-break hotspot determinants
AUTHORS: Yu-Chien Chuang and Gerald R Smith
ARTICLE TYPE: Research Article

Thank you for your recent letter. I understand how disappointed you must feel.

Given the opinions stated by the reviewers, I had no choice but to reject the paper.

However, we are always willing to give authors the chance to defend their manuscripts. In the light of the comments you make in your letter, I have decided to proceed as follows. I would be happy for you to submit a revised version of your manuscript that deals as far as possible with the points raised by the reviewers, together with a detailed rebuttal of any other matter that cannot be settled in the manuscript itself. I will then send the revised version and the rebuttal back to the reviewers. If they are convinced by your arguments, then we would be happy to accept the manuscript for publication.

David Glover
Handling Editor

Letter from Editor-In-Chief (5 November 2021)

Dear Gerry,

I am in communication with Dr Glover and we're still deciding whats best to do to. One of the issues is getting previous reviewers to look at papers again once they have reviewed things twice.

Once we know more you will be hearing from us.

Cheers

Michael

Second revision (1 December 2021)

Cover letter

Dear Dr. Glover,

Thank you for allowing us to resubmit our manuscript "**Dynamic configurations of meiotic DNA-break hotspot determinant proteins**" for publication in the Journal of Cell Science. We have revised it as best we can in accord with the reviewers' suggestions and made the following major changes:

1. We have changed the title, but because the reviewers did not clearly say how it should be revised, we have submitted a list of additional possible titles. We think the title we have used accurately reflects our study of linear element proteins, which are also the protein determinants of DSB hotspots in S. pombe. We are, of course, open to further suggestions.

2. We have removed from the Introduction two paragraphs on crossover interference. We mention interference in two sentences in the Discussion, where we now state that it is weak in S. pombe, but statistically highly significant.

3. We have reduced throughout the manuscript suggestions that the S. pombe linear elements are structurally and functionally similar to the synaptonemal complex of other species. We state at the beginning of the Discussion properties that are, nonetheless, similar.

4. We point out that our conclusion that the linear elements are sensitive to hexanediol is based on our quantification (Figure 6), whereas Ding et al. (2021) said they are not sensitive but did not show quantitative data.

We hope that these changes satisfy the reviewers. If you or they have further specific changes we should make, we would be pleased to do that. Thank you for overseeing the publication of our research.

Yours sincerely,

Gerald R. Smith
Professor, Division of Basic Sciences

Author response to reviewers' comments

Please see our submission of 3 September 2021 with our detailed response to the second reviews. Our response to the third reviews, which we sent to the Editor on 7 October 2021, contained the following (please see our letter of submission with this third revision for additional responses to the reviews and changes in our manuscript, marked in yellow highlight for the second revision and in red font for the third revision):

1. Rev. 2 says, "But the title should reflect what is in the paper." We think it did, but we now have modified the title to make it more precise. Our data here show that the LinE proteins change their configuration during meiosis and therefore justify "Dynamic configurations." Published work (Fowler et al, Mol Cell 2013) shows that the S. pombe LinE proteins are required nearly genome-wide for DSBs at meiotic hotspots and bind to DSB hotspots with high specificity (a point perhaps overlooked by Rev. 2) and therefore justify "meiotic DNA-break hotspot determinant proteins." We have also submitted additional suggested titles if the one we chose is not satisfactory. We would welcome the reviewer's suggestions.

2. Rev. 2 says, "They do not mention that, if interference exists in pombe, is quantitatively weak." We have removed from the Introduction our discussion of crossover interference and mention interference only briefly in the Discussion. S. pombe has highly significant crossover interference (I = 0.26 ± 0.05; 0.24 ± 0.07; 0.31 ± 0.04 in three separate sets of experiments; Fowler et al, PNAS 2018, Tables 1 and S2). We have now stated on page 12 the degree of interference.

3. Rev. 3 says, "This examination is based entirely on imaging, with mechanistic details only inferred." The mechanistic details discussed are based on many previously published data that we cite (for example, Fowler et al, op cit; Davis et al, Curr Biol 2008; Estreicher et al, Curr Genet 2012; Ma et al, Sci Reports 2017; Wintrebert et al, J Cell Sci 2021; Ding et al, Chromosoma 2021). We have added these references to additional points in the manuscript. We think it is proper to use published data in discussing our results.

4. Rev. 3 says, "… but they really do need to base conclusions on what they have demonstrated and not what they imagine to be true." Rev. 2 says, "Speculation need to be bound but what is known." As noted above, our conclusions are based on our data and previously published data that we cited in the manuscript. Our model (Figure 8, "Model for dotty to linear to dotty configurations of LinE structures") uses these data to propose how DSB formation and repair are controlled during meiosis. We clearly label it as a model and distinguish it from our conclusions. As we said in our response to the second reviews, "We think scientific models should incorporate previously reported findings as well as the current findings and that useful models also propose features to motivate further experiments and discoveries." Paul Nurse has amplified this view in his subsequent article "Biology

must generate ideas as well as data" in Nature (https://www.nature.com/articles/d41586-021-02480-z). We hope the reviewers will agree with this philosophy.

Third decision letter (29 December 2021)

MS ID#: JOCES/2021/259061
MS TITLE: Dynamic configurations of meiotic DNA-break hotspot determinant proteins
AUTHORS: Yu-Chien Chuang and Gerald R Smith
ARTICLE TYPE: Research Article

Dear Dr. Smith,

I am happy to tell you that your manuscript has been accepted for publication in Journal of Cell Science, pending standard ethics checks. Where referee reports on this version are available, they are appended below.

Reviewer 1

Advance summary and potential significance to field

I was already satisfied with the previous reply; now, I consider that the authors have also satisfied the requirements from the other reviewers. In my opinion, the manuscript, in its present form, has quality enough and reports conceptual advancements to deserve publication.

Comments for the author

I was already satisfied with the previous reply; now, I consider that the authors have also satisfied the requirements from the other reviewers. In my opinion, the manuscript, in its present form, has quality enough and reports conceptual advancements to deserve publication.

Reviewer 2

Advance summary and potential significance to field

see previous reviews

Comments for the author

The authors have addressed my concerns.

Reviewer 3

Advance summary and potential significance to field

I have no comments beyond my previous review.

Comments for the author

This is at this point, an editorial decision, not a review issue.