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A MatP-divisome interaction coordinates chromosome segregation with cell division in E. coli

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1st Editorial Decision 22 December 2011

Thank you for submitting your manuscript for consideration by The EMBO Journal, and please excuse the delay in its in-depth evaluation due to limited referee availability at this time. We have now received the reports of three expert reviewers, which I am attaching below. As you will see, all referees consider your results on chromosome-divisome interconnections in E. coli interesting and potentially important, and therefore in principle warranting publication in a general journal like ours. At the same time, they however also make clear that the study is in its current state not yet publishable. In particular, all referees take issue with the overall organization and presentation of the study, the tenuous connection between the two halves of the analyses, and the frequent overinterpretation of the finding in the second part of the investigation. Since the basic findings are nonetheless of interest and the raised criticisms could in principle be dealt with, I would like to give you an opportunity to respond to the comments and improve the manuscript in the form of a major revision. However, I should make it clear the extent of the current concerns and criticisms (especially those raised by referee 1) will necessitate a very thorough re-evaluation of the data and their interpretations, as well as major efforts in re-organizing the manuscript and tightening the presentation. I should also point out that it is our policy to allow a single round of major revision only, and that it will therefore be important to diligently and comprehensively answer to all the specific points raised at this stage in the process; including new experiments where appropriate. When revising the manuscript text, please also take care of editorial issues such as proper and complete reference formatting, inclusion of a conflict of interest statement, avoidance of specialist jargon and introduction of special abbreviations (such as 'FROS') already at their first occurrence. I would also appreciate if you would have the manuscript proof-read by a native English speaker before resubmission.

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

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: http://www.nature.com/emboj/about/process.html

Should you have any additional question regarding this decision or your revision, please do not hesitate to contact me. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

Referee #1

The authors present very strong, well-developed evidence that MatP/matS specifically colocalize with the emerging septum at midcell by direct association with ZapB. However, both the Summary and the Discussion emphasize the possible functional significances of this association in envisioned interplay with DNA replication. The data that set up this assertion are very interesting, but they do not provide evidence for the conclusions the authors wish to draw. The paper should be rewritten more conservatively, emphasizing what is clear, presenting the replication-related observations as "findings", and then modestly suggesting a short interpretation in the Discussion.

As a general point, this paper is quite hard to read. This reviewer is at a loss as to suggest fixes, but more focus would help. Further, the paper really presents two stories, not one, with the second story not really fully developed. Perhaps this part could be pared down to the most critical findings.

As another general point, the experiments in this paper use a variety of different growth rates where cell cycles are significantly different. This is extremely confusing and requires the authors to make certain assumptions about what is going on. If this could be minimized, it would be helpful.

Targeting of the Ter MD to the septal ring.
1. The authors say from previous data that "As cells progressed in the cell cycle, the MatP focus migrated to mid-cell..... Snapshot analysis cannot show migration versus dissociation and reassociation. This assumption/complexity should be stated more rigorously.

2. The fact that all cells have a MatP focus appears only at the last line of p6. It is critical for all of the conclusions that appear before that point and should be mentioned earlier.

3. It is unclear that cumulative curves presented are really "cumulative curves" in the sense that information has been integrated ("cumulated") over time. They seem to be simply a plot of cell percentages as a function of cell size. Further, the data are normalized to 100% of the starting values to make the point of interest. To be correct, since Z-rings and MatP foci are both analyzed in the same cells, a better way to show the result is to plot, as a function of cell size, the appearance and disappearance of different classes of cells as defined by 0, 1 central, 1 polar, 2 central MatP foci x presence/absence of FtsZ ring (and, for completeness and to anticipate other conclusions) presence/absence of discernible invagination in the phase images by some standard criterion (which is never specified). These plots should be added to, or substituted for, what is presented.

4. There is ambiguity between p6, text line 7 ("MatP focus split into two") and p6, text lines 17-18 ("cells with two MatP-mCherry foci....one focus near the pole and one focus near midcell").

5. It is unclear why different growth rates were used for different experiments described on p6 (120min vs 70min) This is confusing and compromises unambiguous intepretation of the results. At the very least this should be explained.

6. The logic of the following sentence is incorrect. "We also noticed that the splitting of the MatP focus into two adjacent foci is concomitant with septum constriction, i.e. 96% of cells with a distinct septum displayed two MatP-mCherry foci". To draw the conclusion of interest, it is also necessary to say that cells without a distinct septum always had a single focus and that cells with two foci always appeared in cells with a distinct septum. If the situation is too complex to permit such simple statements, the actual situation should be presented (eg in the bar graphs suggested in (3) above).

7. The fact that MatP overexpresses with aberrant FtsZ foci after overproduction is much stronger evidence for MatP/FtsZ association than the no-casamino acids experiment, which could be taken as evidence against the hypothesis. It would be better to present the overproduction data first (or maybe only present this data). If the additional point is to be made, that MatP association does not require FtsZ, it should be made later. Also, given the extensive discussion of how colocalization with FtsZ is achieved, it would be useful later in the paper to discuss this apparent "exception". Is it leftover septum material, or just ZapB, but not a ring? or?? In fact, if the answer is known, it could be presented after the ZapB results as amplification of this point rather than as an exception to the point the authors wish to make first.

ZapB is the target of MatP at mid-cell

8. p7, second line from bottom. "a dynamic diffused signal...indicting a random distribution of the plasmids moving rapidly in the cellular space". This statement requires time lapse analysis which is not presented. What is clear that there is diffuse localization. Dynamics is not shown and this should be omitted.

9. p8, top eight lines. All of the controls for Figure 2A should be shown, not "data not shown".

ZapA and ZapB are required for the mid-cell targeting of MatP

10. This section is poorly organized. The second sentence ("As expected....") should come first. Then the mutant analysis should be presented.

11. last sentence: it is not shown that functional ZapB is required for interaction with MatP. It is shown that in the absence of ZapA, MatP foci do not colocalize with the aberrantly-localized ZapB. This implies that either the aberrantly localized ZapB is aggregated and not capable of interacting with MatP OR that some other feature of the midcell apparatus is required for MatP localization in addition to ZapB, ie that ZapB is necessary but not sufficient.

Deletion of zapB alters the segregation of the Ter MD

12. English problem: "The localization pattern of MatP-mCherry was altered in the absence of ZapB (Figure) copared to the wild type strain (Figure)." Omit "As described above" - confusing reference.

13. The title of this section announces altered segregation of the Ter MD. What is presented is the localization of MatP, with Ter data in the SI. The title should be changed to "Deletion of zapB alters the localization of MatP foci". The data are somewhat overinterpreted according to the authors' ideas. Qualitatively, there are more MatP foci. This implies that ZapB (and more specifically, presumably, MatP interaction with ZapB and the emerging septum) is required for higher order associations of MatP. The authors wish to interpret this result in a certain way, but their exposition of this interpretation is unclear. Thus: "frequent splitting of the Ter MD according to the replichore localization rule..." It is unclear what the replichore localization rule has to do with this conclusion. Clarify or omit. Futher, aberrant localization of Ter is find, but NONE of these findings pertain to "segregation of the Ter MD". Segregation is a dynamic functional process; what is measured here is localization.

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14. The authors carefully point out that their interpretation of "less prolonged localization" of the Ter MD at mid-cell requires that replication timing must be unperturbed in zapB. It would be helpful to have some data which suggests that this assumption is valid.

15. The authors do not really emphasize the fact that even in zapB, there are many cells with a single MatP focus at midcell. This might be linked to the data above suggesting that FtsZ is not absolutely required for midcell localization. Clearly, the story is more complex than simple binding of MatP to FtsZ/ZapB and this complexity should be discussed.

Deletion of zapB does not influence the condensation of the Ter MD.

16. It is clear that ZapB is required for reduced mobility of Ter MD markers and not for inter-locus distances in the Ter MD. The latter finding straightforwardly implies no role for "condensation". The former finding is less clearly interpretable. The rates of movement indicated are too slow to reflect "apparent diffusion coefficients" and thus should reflect overall chromosome dynamics. There is no specification of the nature of the movement, ie is it directed or not, etc. Certainly, since ZapB is involved in anchoring the Ter MD, it should restrict movement at some points in the cell cycle, but the data presented are for all cells at all stages. Wouldn't it be possible to separate things out better? For example, in zapB cells, there are still many one-MatP focus cells with the focus at mid-cell. Do these cells show less mobility?

The nucleoid segregation is dramatically altered in the matP zapA or matP zapB strains.

17. Why were these experiments done in LB when all of the above experiments were done under much slower growth conditions? Is DAPI staining only possible in LB? This seems unlikely.

18. The fact that the two mutations in these double mutants give synergistic effects implies that ZapA and ZapB have roles other than to anchor MatP, which is not surprising, but also that in a zapAB background, absence of MatP has a big effect. This shows that MatP is relevant even in the absence of ZapB. This is not very surprising, is it? Maybe this data could be omitted. Or else deeper significance discussed to motivate the presentation?

Potential of MatP to organize and target the Ter MD at mid-cell.

19. The idea to use linear chromosomes to analyze the Ter MD is very nice and the use of differently-colored tags to see colocalization is excellent. This is very clear and interesting data.

The terminus regions of linear chromosomes are replicated at mid-cell.

20. It is not specified in the text which growth conditions were used for these experiments.

21. The authors are struck by the finding that, with both linear and circular chromosomes, a single SSB focus is seen at midcell at the "predicted time of the two termini replication". They are thinking that the Ter MD is already localized to midcell by the time replication reaches that region. They then examine colocalization of Ter2 and SSB foci in a linear strain. They state that "at the moment of its replication, the Ter-2 locus must be colocalized with an SSB focus". This is true. But it is also true that colocalization of Ter2 and SSB could occur for other reasons. If the TerMD has been brought to midcell, and one replication fork has already stopped at a Ter/Tus site, colocalization does not mean that Ter2 is being replicated but only that a terminus-associated single-stranded DNA region is present. The authors say that:
"These observations demonstrated that the replication of a large terminus region, [250kb before dif] is achieved at midcell for most cells with a linear chromosome. Therefore Ter MD replication occurs at mid-cell independently of chromosome circularity." This is likely an accurate conclusion, for these conditions, despite certain assumptions about exactly what is going on, and it is striking for exactly the reasons the authors suggest, i.e. that the timing of localization of a Ter segment to midcell does not require that it be linked to the other half of a circular chromosome, thus raising the question of how this localization is achieved.

Replication triggers the migration of the TerMD to midcell.

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22. The authors suggest that movement of terminus region(s) to midcell is an "active and regulated process that influences chromosome positioning". It is unclear what this means. If it simply means that having bidirectional replication of a circle is insufficient, well, this was already true. Bidirectional replication will not put the terminus region at midcell unless there is a tendency for newly-replicated regions to move away from one another in a particular way. So the "prior model" needs to be specified in more detail.
The authors data interestingly show that some aspect of chromosome dynamics moves terminus regions to midcell even if the two halves of ter are not connected. And that mechanism presumably will not simply move the terminus regions but will, in some way, also be involved in overall chromosome disposition. It could be noted that, since the available evidence suggests that the genome is linearly displayed within the cell anyway, even with a circular chromosome, it is (in retrospect and in this context) "not surprising" that a linear chromosome behaves like a circular one. This could be considered. In fact, the authors might wish to consider carrying out the experiments in this section on a linearized chromosome. This reviewer is willing to bet that nothing will change.

23. Growth conditions are critical. They should be stated in the text. Also, the images present "representative cells". This should be made clearer and some numbers provided as to exactly the types of cells seen. This is particularly true since terminus region loci can exist at either end of the cell, with variations from cell to cell.

24. The first experiment performed in this section asks about the dynamic behaviors of two loci separated by 200kb. This is, in chromosome space, a really short distance. The fact that the two loci exhibit such dramatically different localization is therefore quite peculiar. Moreover, the description in the text does not accurately convey the fact that MatP signal is seen extending between two widely-separated loci. This obviously contributes to the authors' model, but the relevant points are not explicitly made. Rather the authors emphasize the "correlation with the genetic map". For this reviewer, this is possibly relevant but secondary. Further, this information is not integrated with the complexities that derive from linear chromosome organization.

25. Given that DNA replication is likely the driving force for chromosome dynamics by any mechanism, it is not surprising that DNA replication is relevant to the chromosome dynamics described in this paper, as shown by the presented dnaBts experiment. But the conclusion that "replication triggers the migration of the TerMD to mid-cell" is a vast overstatement. Further, why were these experiments done with Ter3 instead of with the Ter 6/7 pair used in the preceding studies, which would have been more informative.

26. Chromosome rearrangement analysis is simply too complex to be informative. This reviewer would omit it. Any result is possible.

27. The model (Figure 7) should not appear until the Discussion, rather than at the end of the Results. Moreover, the data do not under any circumstances support the conclusion that "localization of the replication machinery is an active and regulated process that involves a gathering of replisomes replicating the Ter region independent of the chromosome linear or circular configuration".

Discussion

The authors are free to say whatever they wish in the Discussion, but in the opinion of this reviewer, nearly everything in the Discussion is an unwarrented overinterpretation of the data. The authors think that they are "on to something". This is fine. Maybe so. But the current data do not lead critically to any particular model. This reviewer could make a completely different one, and could argue against that of the authors. The Discussion should focus on the more straightforward parts of the paper and the speculation should be kept to a minimum.

The title must be changed

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Referee #2

The report by Espeli and co-workers investigates the dynamic localization of an important macrodomain of the bacterial chromosome (the terminus) and the dependence of its localization on the organization protein, MatP, the division machinery, and the replication process. The authors show that MatP and the terminus region localize to midcell at about the time the FtsZ ring is formed. They further investigated the role of division proteins in anchoring the terminus region at midcell and found that this is achieved by an interaction between MatP bound at matS sites in the terminus region and the septal ring protein ZapB. Careful analysis of the phenotype of zapB mutants revealed that the MatP-ZapB interaction is responsible for the reduced mobility and specific subcellular localization of the Ter region, but not the condensation/organization of the Ter region (based on inter-focal distance between two Ter markers). Thus, MatP appears capable of organizing the Ter region independently of its ability to anchor the region at the septal ring. During this investigation it was noted that the Ter region was capable of being recruited to midcell in the absence of MatP but not anchored there for extended periods of time. The authors therefore investigated the role of the replication process in the migration of the Ter region to midcell and, using a clever linearization method, whether or not chromosome circularity was required for this migration. Interestingly, it was found that the Ter region migrated to midcell even when it was split by the linearization process. Furthermore, the authors showed that replisomal foci were recruited to midcell as the terminus was replicated and that this did not depend on the circularity of the chromosome (i.e. it was not merely the consequence of two replisomes meeting at the Ter region as they move around a circular "track"). This finding suggests that the recruitment of replisomes to midcell during Ter replication is dependent on a specific structural determinant that has yet to be identified.

Overall, the experiments described in this paper are convincing and interesting. My only major criticisms are: (i) The paper was written in such a way that the two parts are not well connected. The MatP-ZapB portion of the paper seems very disconnected from the replication/Ter migration portion of the paper. A better connection might be achieved if the paper was reorganized to cover the topics in the order that they occur during the cell cycle. First describe the overall observation that the Ter region first migrates to midcell and that this required replication, and second describe that after migrating to midcell the Ter region is retained or anchored to this region by the MatP-ZapB interaction. This organization may also help add in experiments that test the possible role of ZapB or other components of the septal ring in bringing the replisomes to midcell during Ter replication. (ii) More effort should have been made to proofread the manuscript for clarity and grammatical errors/typos.

Major Specific Comments:

1) Although the results in Figure 3D-E suggest that ZapB is not required in the migration of the Ter region to midcell (just its anchoring there), it may be required for the movement of replisomes to midcell. This was not tested directly, and would be nice to include since it would help connect the two sections of the paper, and because of the finding that ZapB may play additional roles in nucleoid segregation on top of its interaction with MatP (see comment 7).

2) ZapB- mutants do not display gross nucleoid defects, suggesting that either the anchoring of Ter to the septal ring is not important or that another process compensates for the lack of anchoring in ZapB- cells. This should be discussed.

3) The last two sentences of the introduction, " Finally, we have shown that MatP is not responsible for the migration of the Ter MD from the pole to midcell. Instead, we present strong evidence suggesting that the capture of the Ter MD is promoted by the replisome, which is then localized at midcell, and attracts DNA", make little sense to me. Do you mean, "Instead, we present strong evidence that Ter DNA is recruited to midcell during the process of replication by midcell localized replisomes"?

4) At the end of the first results section it cannot be concluded that, "FtsZ is not the only determinant controlling the process". At this point it is only clear that MatP is recruited to Z-rings. Before additional results are presented it is formally possible that a direct FtsZ-MatP interaction is all that is required for the recruitment.

5) Care should be taken when using the word "targeting". This implies "delivery", when in most instances I think you mean "anchoring". Also, care should be taken when using "capture" with respect to the replisome and the Ter region. In the context of this paper "capture" is very similar to "anchoring". I would instead refer to the replisome being involved in "recruiting" the Ter MD to midcell as opposed to "capturing" it.

6) In Figure 2D, why is there a mixture of white and blue colonies on the plates that test for a 2-hybrid interaction between MatP and ZapB? Shouldn't they all be blue?

7) In figure 3 I-L a MatP- Zap+ control micrograph should be presented so the reader can compare the double mutant phenotypes to the single mutants. It is not clear why the double MatP- ZapB- strain should display an elevated nucleoid structure/segregation defect over the MatP- strain alone. MatP- mutants are presumably defective for Ter organization and anchoring to the septal ring. If the only role of ZapB is the anchoring of Ter to the septal ring (via MatP), then MatP- and MatP- ZapB- mutants should be equivalent. This suggests that ZapB has an additional role in nucleoid segregation and is mentioned in the Discussion. It would be helpful to the reader if this was acknowledged in the Results section along with "see Discussion" to indicate that this will be discussed later.

8) Figure 3E-F: The extra foci in the images were confusing. At first I though they represented some analysis of the foci from the outlined cell. I then realized that they are presumably ter loci in another cell adjacent to the cell the authors have highlighted. Please either crop the images to only display the cells you want to discuss in the image, or explain in the legend that the foci next to the numbers are from an adjacent cell.

9) In the discussion the authors state that "bacterial two-hybrid assay revealed a direct ZapB-MatP interaction". Since the assay was performed in E. coli, it cannot be definitively ruled out that the interaction is indirect. Further in vitro work is required to determine if the interaction is direct or not.

Minor Specific Comments:

1) Several references appear not to be formatted appropriately in the text.

2) Bottom of page 3, it is not clear to me what a "transversal sub-cellular organization" is. I think you mean that the chromosome can serve as a scaffold for subcellular organization along the long axis of the cell.

3) Beginning of results section: do you mean a significant percentage of the cell cycle as opposed to a "persistent step"?

4) Figure 1G and associated text: The figure does not show a frequent localization of MatP-mCherry foci with non-medial Z-rings. It would be nice to know the frequency of MatP foci co-localized with medial versus non-medial Z-rings to see if there is a bias for MatP localization to medial rings.

5) Bottom of page 7: "The system consisted of..." should read something like, "To test this, we constructed a system....."

6) In figure legends it is commonly written, "...and phase contrast microscopy (grey) of the MG1655 cells grown...". It would be better to say, "...and phase contrast micrographs of MG1655 cells grown..."

7) Results page 8; It is better to use "inactivation" rather than "inhibition" when referring to the behavior of TS mutants at the non-permissive temperature.

8) Given the subcellular localization results in Fig. 3, it would be interesting to test whether the two-hybrid interaction between MatP and ZapB requires cellular ZapA. It may provide further support for the idea that ZapB must be associated with the septal ring in order to interact with MatP.

9) Line numbers are helpful for the review process. Please include them next time.

Referee #3

This paper further examines the role of MatP in localizing and organizing the terminus region of the E. coli chromosome. Although MatP was previously identified and shown to play such a role, an understanding of how MatP and the Ter macrodomain are localized to and maintained at mid-cell were unclear. Here, the authors begin by showing that the localization of MatP to mid-cell is often coincident with the localization of FtsZ to mid-cell. The localization of a matS-containing plasmid depends on ftsZ as well as two FtsZ-ring stabilizing proteins, ZapA and ZapB, with the latter possibly interacting directly with MatP (see below for more on this issue). This interaction between ZapB and MatP appears important for the localization of the Ter macrodomain to mid-cell, for Ter macrodomain condensation, and chromsome segregation. Using a clever trick for generating linear chromosomes, the authors split the Ter macrodomain, but show that it still assembles into a single focus in most cells, likely due to the action of MatP. The final part of the paper presents data indicating that the initial translocation of MatP from the poles to mid-cell depends on replication. In sum, the authors have provided quite a bit of new and interesting data on how MatP functions. Although many of the conclusions are well supported, there are a few issues that need to be addressed. In particular, the authors need to further substantiate their model that ZapB directly binds MatP to retain it at mid-cell.

Issues/Concerns:

Fig. 1G: The authors overexpress ftsZ and claim that MatP frequently colocalizes with the now aberrantly localized FtsZ. The single panel shown in 1G is difficult to assess. A proper quantification of both FtsZ and MatP localization would be helpful.

p. 8: The authors indicate that the plasmid containing matS sites does not localize in a matP null strain. These data, with quantification, need to be shown in the paper as they represent a critical control.

Fig. 3F: The authors show that ZapB mislocalizes in a zapA mutant, but oddly MatP doesn't colocalize anymore with ZapB. These data appear to conflict with the notion that MatP binds ZapB. The authors suggest that the ZapB foci in the zapA mutant are simply non-functional aggregates, but there's no data to substantiate such an assertion. I'm left a little uneasy about the conclusion that MatP binds ZapB for this reason and that indicated below.

Some of the authors' data supports the notion that ZapB binds MatP to retain it and the associated Ter macrodomain at mid-cell. However, this conclusion rests heavily on the finding that MatP is mislocalized in the ZapB mutant. Given that a zapB deletion causes many defects, the influence of ZapB on MatP localization could be indirect. The bacterial two-hybrid data helps support a direct relationship, but is insufficient without additional evidence. In particular, the authors need to identify a mutation in ZapB that specifically disrupts the binding of MatP in two-hybrid analysis, but does not prevent ZapB from fulfilling its other FtsZ/divisome-based roles. If such a mutant does not disrupt cell division or FtsZ/divisome ring formation, but does disrupt MatP/matS localization, I think their model will stand on firmer ground. This experiment is essential. The authors should also provide additional evidence for direct binding in the form of co-IP given how heterogeneous the colonies are in the two-hybrid analysis (which leaves me concerned that the interaction is not specific or meaningful).

Fig. 6B: The authors must quantify their analysis of Ter localization in the dnaBts mutant. In addition, I think it is imperative that the authors confirm these results by measuring Ter localization after perturbing DNA replication in another, independent way.

The authors must reference Lau et al. (Mol. Micro., 2003) in describing the localization pattern of ter-proximal regions using MatP-mCherry localization. The work here essentially recapitulates previous FROS-based analyses of Ter localization in slow-growing cells published by the Sherratt group.

The authors need to address whether their findings extend beyond the MG1655 background. The

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relevance of the work depends on the generalizability of the results, so at least some of the key experiments should be verified in other commonly used E. coli strains.

1st Revision - authors' response 19 March 2012

Detailed description of the responses to the referees.

Referee 1:

1)The authors present very strong, well-developed evidence that MatP/matS specifically colocalize with the emerging septum at midcell by direct association with ZapB. However, both the Summary and the Discussion emphasize the possible functional significances of this association in envisioned interplay with DNA replication. The data that set up this assertion are very interesting, but they do not provide evidence for the conclusions the authors wish to draw. The paper should be rewritten more conservatively, emphasizing what is clear, presenting the replication-related observations as "findings", and then modestly suggesting a short interpretation in the Discussion.

We agree with the referee 1 that the experiments presented in the manuscript did not directly demonstrate a capture of the Ter MD by the replication factory. We have rewritten the manuscript more conservatively describing our findings, tightened some paragraphs and transferred every interpretation to the discussion. To reinforce the link between replication and Ter MD localization, we performed another experiment that is now described on Figure 3F (**page 10 line 26 – page 11 lines 1-4**; see details further below)

2) *As a general point, this paper is quite hard to read. This reviewer is at a loss as to suggest fixes, but more focus would help. Further, the paper really presents two stories, not one, with the second story not really fully developed. Perhaps this part could be pared down to the most critical findings.*

We believe that the new organization has considerably improved the manuscript. The part on the association between replication and migration of the Ter MD has been pared down to the most critical findings as suggested.

3) *As another general point, the experiments in this paper use a variety of different growth rates where cell cycles are significantly different. This is extremely confusing and requires the authors to make certain assumptions about what is going on. If this could be minimized, it would be helpful*

This aspect has been clarified, every experiment involving subcellular localization are now performed in the same minimal medium supplemented with glucose and casaminoacids. The generation time of the cells grown in liquid culture for the snapshot analysis (70 min) and the cells grown on the microscope stage on agar pad (~110 min) for the timelapse experiments are different. This is because the liquid cultures were performed at 30°C and the timelapses at 25°C. We (Espéli et al 2008) and others have shown that the length of each step is proportionally modified according to the generation time; the course of the different steps of the cell cycle is not changed by the temperature. This is clearly stated in the Manuscript and in the legend of the figures. The only experiments performed in LB are the one described on **Figure 6J-N** (**text page 16, lines 1-11**); the reason for this change is because the filamentation of the *matP* strain is only detectable in LB.

4)*The authors say from previous data that "As cells progressed in the cell cycle, the MatP focus migrated to mid-cell..... Snapshot analysis cannot show migration versus dissociation and reassociation. This assumption/complexity should be stated more rigorously.*

We agree with referee 1 that it was not possible from the previous data cannot distinguish between migration versus dissociation and reassociation. We have reformulated more precisely the sentence (**page 6, lines 6-7**). Experiments reported now **(Figure 1 and Figure S1**, text **page 6 lines 15-21**) measuring the fluorescence intensity in cells with two foci at the pole and at mid-cell revealed an inverse relationship between the amount of fluorescence in the polar foci and the central foci. This experiment together with Figure 3 that showed that the Ter MD migrates progressively to mid-cell starting from its *oriC* proximal part showed that the MatP focus does not migrate as a whole from

the pole to mid-cell but rather that MatP molecules dissociated progressively from the Ter region at the pole and reassembled at mid-cell after migration of the Ter DNA This is now clearly indicated in the text **page 10, lines 7-17**.

5*) The fact that all cells have a MatP focus appears only at the last line of p6. It is critical for all of the conclusions that appear before that point and should be mentioned earlier.*

The fact that every cell present at least one MatP focus is now presented at the beginning of the paragraph.

6) *It is unclear that cumulative curves presented are really "cumulative curves" in the sense that information has been integrated ("cumulated") over time. They seem to be simply a plot of cell percentages as a function of cell size. Further, the data are normalized to 100% of the starting values to make the point of interest. To be correct, since Z-rings and MatP foci are both analyzed in the same cells, a better way to show the result is to plot, as a function of cell size, the appearance and disappearance of different classes of cells as defined by 0, 1 central, 1 polar, 2 central MatP foci x presence/absence of FtsZ ring (and, for completeness and to anticipate other conclusions) presence/absence of discernible invagination in the phase images by some standard criterion (which is never specified). These plots should be added to, or substituted for, what is presented.*

The cumulative curves have been replaced by plot showing the cell percentages as a function of cell size as suggested by referee 1. Invagination was monitored thanks to a line scan analysis of the phase contrast images (**Figure 4B** and **text page 12, lines 1-5**). We empirically determined that a 20% reduction of the phase signal, measured at mid cell, was the threshold to consider invagination. This is now indicated in the materiel and methods.

7) *There is ambiguity between p6, text line 7 ("MatP focus split into two") and p6, text lines 17-18 ("cells with two MatP-mCherry foci....one focus near the pole and one focus near midcell").*

We have cleared this ambiguity by choosing to limit the usage of duplication to the event happening before cell division to indicate the appearance of two sister loci (i.e. when MatP focus become 2 foci on each side of the constricting septum (**page 6 line 8**, **page 12 line 25**) We used “split” to characterize the event happening on one chromosome (i.e when the TerMD is observed as two foci, one polar and one central in the newborn cells, **page 10 line 8** (splitting of Ter-6 and Ter-7 loci)) and in the context of the linear chromosome when the linearization process splits into two part the Ter MD (**page 2 line 10**, **page 6 line 25**, **page 6 line 26**)). We used separation to indicate the separation of different entities, **page 18, line 13** (separation of FtsZ and MatP)).

8) It is unclear why different growth rates were used for different experiments described on p6 (120min vs 70min) This is confusing and compromises unambiguous intepretation of the results. At the very least this should be explained.

We clarified this point. See point 3

9) The logic of the following sentence is incorrect. "We also noticed that the splitting of the MatP focus into two adjacent foci is concomitant with septum constriction, i.e. 96% of cells with a distinct septum displayed two MatP-mCherry foci". To draw the conclusion of interest, it is also necessary to say that cells without a distinct septum always had a single focus and that cells with two foci always appeared in cells with a distinct septum. If the situation is too complex to permit such simple statements, the actual situation should be presented (eg in the bar graphs suggested in (3) above)

A bar graph is now presented on figure 4B, it describes the percentages of cells with a polar MatP focus and no FtsZ ring, a polar MatP focus and a central ftsZ ring, a central matP focus colocalized with an FtsZ ring and two MatP foci flanking the FtsZ ring. The describtion of the result has been modified according to referee 1 suggestion. “Interestingly, MatP followed FtsZ dynamics, i.e. small cells without an FtsZ ring presented a MatP focus near the pole, cells with a mid-cell MatP focus always presented a colocalized FtsZ ring, the largest cells presented two MatP foci flanking the FtsZ ring (**Figure 4B**). We noticed that the duplication of the MatP focus into two adjacent foci is concomitant with septum constriction; i.e., 96% of cells with a distinct septum displayed two MatP-mCherry foci.” (**page 12 lines 2-7**)

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10) The fact that MatP overexpresses with aberrant FtsZ foci after overproduction is much stronger evidence for MatP/FtsZ association than the no-casamino acids experiment, which could be taken as evidence against the hypothesis. It would be better to present the overproduction data first (or maybe only present this data). If the additional point is to be made, that MatP association does not require FtsZ, it should be made later. Also, given the extensive discussion of how colocalization with FtsZ is achieved, it would be useful later in the paper to discuss this apparent "exception". Is it leftover septum material, or just ZapB, but not a ring? or?? In fact, if the answer is known, it could be presented after the ZapB results as amplification of this point rather than as an exception to the point the authors wish to make first.

We agree with the referee 1; the overexpression experiment is a much stronger evidence for MatP/FtsZ association. We removed the no-casamino acids experiment from figure 4. As suggested by the referee 2 and 3, we also reorganized the figure describing the overexpression experiment to make the interpretation easier for the reader (text from **page 11 line 24 to page 12 line 17**).

11. p7, second line from bottom. "a dynamic diffused signal...indicting a random distribution of the plasmids moving rapidly in the cellular space". This statement requires time lapse analysis which is not presented. What is clear that there is diffuse localization. Dynamics is not shown and this should be omitted. 9.
p8, top eight lines. All of the controls for Figure 2A should be shown, not "data not shown".

The timelapses experiments and the corresponding kymograph for the dynamics of the pGB2parST1 and pGB2parSt1-2matS plasmids are now presented on supplementary Figure S7 (text from **page 12 line 24 to page 13 line 5**). The localization and dynamics of the pGB2parST1-2matS plasmid performed in the wt, *matP*, *zapB* strains are now presented on supplementary **Figure S7** (**page 13 line 4-5**).

12) 10. This section is poorly organized. The second sentence ("As expected....") should come first. Then the mutant analysis should be presented.

We have modified the text as suggested (**page 14, line 7**)

13) last sentence: it is not shown that functional ZapB is required for interaction with MatP. It is shown that in the absence of ZapA, MatP foci do not colocalize with the aberrantly-localized ZapB. This implies that either the aberrantly localized ZapB is aggregated and not capable of interacting with MatP OR that some other feature of the midcell apparatus is required for MatP localization in addition to ZapB, ie that ZapB is necessary but not sufficient.

We have modified this paragraph. We have now performed two hybrid experiments in a *zapA* mutant strain; the ZapB-MatP interaction detected by the two hybrid requires ZapA. The cell biology experiment and the two hybrid experiment in the zapA mutant confirmed that ZapA is required to observe the MatP-ZapB interaction. We have now indicated that these results showed that MatP interacts with ZapB when associated in a larger complex comprising at least ZapB, ZapA and FtsZ (**page 14 lines 18-19**).

14) English problem: "The localization pattern of MatP-mCherry was altered in the absence of ZapB (Figure) copared to the wild type strain (Figure)." Omit "As described above" - confusing reference.

We have modified the text as suggested (**page 14, line 24**).

15) The title of this section announces altered segregation of the Ter MD. What is presented is the localization of MatP, with Ter data in the SI. The title should be changed to "Deletion of zapB alters the localization of MatP foci". The data are somewhat overinterpreted according to the authors' ideas. Qualitatively, there are more MatP foci. This implies that ZapB (and more specifically, presumably, MatP interaction with ZapB and the emerging septum) is required for

higher order associations of MatP. The authors wish to interpret this result in a certain way, but their exposition of this interpretation is unclear. Thus: "frequent splitting of the Ter MD according to the replichore localization rule..." It is unclear what the replichore localization rule has to do with this conclusion. Clarify or omit. Futher, aberrant localization of Ter is find, but NONE of these findings pertain to "segregation of the Ter MD". Segregation is a dynamic functional process; what ismeasured here is localization.

We agree with the referee, snapshot analysis does not directly describe segregation. To our knowledge, we have published the only analysis of the segregation pattern of several loci from the E. coli chromosome by using live cells time-lapse analysis (Espeli et al 2008). Our expertise shows that snapshot reveals the major steps of segregation and it is particularly efficient to detect earlier than expected segregation. We do not “wish to interpret our experiments in a certain way” as suggested by the referee. In the previos version of the MS, we did not include the timelapses experiments to simplify the presentation. The segregation pattern of the Ter-6 locus in the absence of ZapB is now presented on the montage and the graphs onF**figure 6H** (text from **page 14 line 22 – page 15 line 12**). It revealed that segregation was indeed affected at least on two aspects: the segregation happened earlier in the cell cycle and the segregation path was less precise. We kept the title.

16) The authors carefully point out that their interpretation of "less prolonged localization" of the Ter MD at mid-cell requires that replication timing must be unperturbed in zapB. It would be helpful to have some data which suggests that this assumption is valid.

We estimated the timing of replication in the *zapB* mutant strain by two methods, first using the SSB-YPet fusion et second by flow cytometry experiments (rifampicine – cephalexine run out). We did not detect important changes in the amount of origins present in the cells compared to the wt. This suggested that there is not any strong alteration of the replication program in the *zapB* strain and is indicated **page 15 lines 9-12** as data not shown.
The increase in the amount of TerMD foci and the earlier segregation of the Ter-6 foci, observed in this strain, could not be explained by a small change in the replication program. Therefore we propose that they must be explained by loss of the sister chromatid colocalization.

17) The authors do not really emphasize the fact that even in zapB, there are many cells with a single MatP focus at midcell. This might be linked to the data above suggesting that FtsZ is not absolutely required for midcell localization. Clearly, the story is more complex than simple binding of MatP to FtsZ/ZapB and this complexity should be discussed

We have added a paragraph about this aspect in the discussion (**page 19 lines 13- 16**).

18) It is clear that ZapB is required for reduced mobility of Ter MD markers and not for inter-locus distances in the Ter MD. The latter finding straightforwardly implies no role for "condensation". The former finding is less clearly interpretable. The rates of movement indicated are too slow to reflect "apparent diffusion coefficients" and thus should reflect overall chromosome dynamics. There is no specification of the nature of the movement, ie is it directed or not, etc. Certainly, since ZapB is involved in anchoring the Ter MD, it should restrict movement at some points in the cell cycle, but the data presented are for all cells at all stages. Wouldn't it be possible to separate things out better? For example, in zapB cells, there are still many one-MatP focus cells with the focus at mid-cell. Do these cells show less mobility?

The referee points out an important aspect. We are still exploring the variation of the dynamic properties of the chromosome during the cell cycle. We believe that this aspect is not essential for this manuscript, and therefore we removed the experiment. The alteration of the condensation is now only illustrated by the Ter-3 –Ter-6 interfocal distance (**Figure 6I**, text **page 15 lines 21-22**).

19. Why were these experiments done in LB when all of the above experiments were done under much slower growth conditions? Is DAPI staining only possible in LB? This seems unlikely

This experiment was performed in LB because filamentation and anucleate cells can only be detected in LB in the *matP* mutant (Mercier et al 2008).

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20. The fact that the two mutations in these double mutants give synergistic effects implies that ZapA and ZapB have roles other than to anchor MatP, which is not surprising, but also that in a zapAB background, absence of MatP has a big effect. This shows that MatP is relevant even in the absence of ZapB. This is not very surprising, is it? Maybe this data could be omitted. Or else deeper significance discussed to motivate the presentation?

We have added a paragraph in the discussion taking into account this synergistic effect (**page 19 lines 19-25**) and the Ter MD condensation that was not altered in the *zapB* mutant (**page 19 lines 5-9**). These observations suggest, as proposed by referee 1, that MatP is relevant even in the absence of ZapB.

21) It is not specified in the text which growth conditions were used for these experiments.

See point 3, the growth medium is now indicated in the legend.

21. The authors are struck by the finding that, with both linear and circular chromosomes, a single SSB focus is seen at midcell at the "predicted time of the two termini replication". They are thinking that the Ter MD is already localized to midcell by the time replication reaches that region. They then examine colocalization of Ter2 and SSB foci in a linear strain. They state that "at the moment of its replication, the Ter-2 locus must be colocalized with an SSB focus". This is true. But it is also true that colocalization of Ter2 and SSB could occur for other reasons. If the TerMD has been brought to midcell, and one replication fork has already stopped at a Ter/Tus site, colocalization does not mean that Ter2 is being replicated but only that a terminus-associated single-stranded DNA region is present. The authors say that:
"These observations demonstrated that the replication of a large terminus region, [250kb before dif] is achieved at midcell for most cells with a linear chromosome. Therefore Ter MD replication occurs at mid-cell independently of chromosome circularity." This is likely an accurate conclusion, for these conditions, despite certain assumptions about exactly what is going on, and it is striking for exactly the reasons the authors suggest, i.e. that the timing of localization of a Ter segment to midcell does not require that it be linked to the other half of a circular chromosome, thus raising the question of how this localization is achieved.

The experiments described in this version of the MS clearly showed that the Ter MD migrates progressively to the mid-cell, starting for the loci replicated the first and finishing by the loci replicate the last (**Figure 3 and Figure S5**, **page 9 from line 19 to page 11 line 15**). They also showed that loci of the Ter MD are localized with SSB foci at mid-cell (**Figure 2**, from **page 8 line 9 to page 9 line 16**).
The referee has probably misunderstood the experiment. It was performed in a strain with a linear chromosome; therefore, replication can not be stopped by the Ter/Tus site because the replication arms are cut, at the tos site, in the replication fork trap. We have added a sentence to explain this point in the text.
The point of this manuscript was to describe the segregation of the Ter MD and not to identify the factors involved in replication machinery localization. This is well beyond the scope of this work. We agree with the referee 1, we still do not know how this localization is achieved and how it could influence migration of the Ter MD.

22. The authors suggest that movement of terminus region(s) to midcell is an "active and regulated process that influences chromosome positioning". It is unclear what this means. If it simply means that having bidirectional replication of a circle is insufficient, well, this was already true. Bidirectional replication will not put the terminus region at midcell unless there is a tendency for newly-replicated regions to move away from one another in a particular way. So the "prior model" needs to be specified in more detail.

We agree with the referee that this paragraph was unclear. The title has been changed (Thje migration of the Ter MD to mid-cell is coordinated with its replication) and the text modified as follow (**page 9 lines 19-24**): "The ends of each chromosome's arm of a linear chromosome were brought to mid-cell at the time of their replication. This observation suggests that in cells with a circular chromosome, the merging of the replisomes foci in one focus was not solely the consequence of two forks running in the opposite orientation on a circular track and meeting at the

terminus already localized at mid-cell. An alternative hypothesis stipulates that the two replisomes could meet at mid-cell before replicating the Ter MD and therefore could provoke Ter MD migration toward mid-cell for its replication."

***23) The authors data interesting**ly show that some aspect of chromosome dynamics moves terminus regions to midcell even if the two halves of ter are not connected. And that mechanism presumably will not simply move the terminus regions but will, in some way, also be involved in overall chromosome disposition. It could be noted that, since the available evidence suggests that the genome is linearly displayed within the cell anyway, even with a circular chromosome, it is (in retrospect and in this context) "not surprising" that a linear chromosome behaves like a circular one. This could be considered. In fact, the authors might wish to consider carrying out the experiments in this section on a linearized chromosome. This reviewer is willing to bet that nothing will change.*

We do not understand the point made by the referee; these experiments have been performed with strains carrying a linearized chromosome or with a circular chromsome.

23. Growth conditions are critical. They should be stated in the text. Also, the images present "representative cells". This should be made clearer and some numbers provided as to exactly the types of cells seen. This is particularly true since terminus region loci can exist at either end of the cell, with variations from cell to cell.

The growth conditions are described in the point 3 and in the materiel and method. We have added a quantification of the number of cells in each category (**Figure 3B** and text **page 10 line 7 to line 11**).

24. The first experiment performed in this section asks about the dynamic behaviors of two loci separated by 200kb. This is, in chromosome space, a really short distance. The fact that the two loci exhibit such dramatically different localization is therefore quite peculiar.

We have added a quantification of the number of cells in each category (se above). Actually, the cells where the two loci exhibit a dramatic different localization represent only 4% of the population. This amount is in the range with the time required to replicate 200 kb (ie 4 min, 10% of C period, 6% of the generation time)

25) Moreover, the description in the text does not accurately convey the fact that MatP signal is seen extending between two widely-separated loci. This obviously contributes to the authors' model, but the relevant points are not explicitly made. Rather the authors emphasize the "correlation with the genetic map". For this reviewer, this is possibly relevant but secondary. Further, this information is not integrated with the complexities that derive from linear chromosome organization.

As the referee 1 pointed out, the MatP signal extends between two foci on the first cell of the Figure 3B. As shown on the second cell, this extension is not always seen. We believe that super resolution microscopy might be needed to clarify this aspect. This is beyond the goal of this manuscript. We have now provided on figure S1 a quantification of the amount of fluorescence in the polar MatP focus compared to the mid-cell focus. It shows that these amounts are inversely proportional. The experiments presented on figure 3 were all performed with a strain with a circular chromosome. Similar experiments performed with a strain with a linear chromosome gave similar results (data not shown).

26) Given that DNA replication is likely the driving force for chromosome dynamics by any mechanism, it is not surprising that DNA replication is relevant to the chromosome dynamics described in this paper, as shown by the presented dnaBts experiment. But the conclusion that "replication triggers the migration of the TerMD to mid-cell" is a vast overstatement. Further, why were these experiments done with Ter3 instead of with the Ter 6/7 pair used in the preceding studies, which would have been more informative.

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We agree with the referee 1 these experiments did not show that the replication itself triggered the migration of the Ter-MD to mid-cell. The title of the paragraph has been modified:" The migration of the Ter MD to mid-cell is coordinated with its replication**"**
The *dnaBts* experiment could not be easily performed with the Ter-6 Ter-7 strain for technical reasons. Because of the MD organization and the effect of MatP, we are confident that any point of the Ter MD is equivalent. Moreover, we have now added an experiment with a *dnaCts* allele, in the way to block replication in a different way as suggested by referee 3 (Figure 3F, text from **page 10 line 25 to page 11 line 4**). This experiment was performed with the Ter-7 tag.

26. Chromosome rearrangement analysis is simply too complex to be informative. This reviewer would omit it. Any result is possible.

We and other have obtained a number of important data about chromosome organization with chromosome rearrangements (Valens et al., 2004; Esnault et al 2007; Thiel et al., in press). In the two experiments described here, the rearrangements have no important physiological consequences. The first experiment consisted in a transposition (without any inversion of the orientation of the DNA compared to replication). The second experiment consisted in a small inversion of a small region carrying a Ter/Tus site to displace the replication fork trap.
We believe that the analysis, limited to the positioning of the Ter MD, is genuine. With the objective to stick with referee 1 suggestion to pared down this part to the most important observations, we transferred these two experiments to the supplementary material (**Figure S6** and **text page 11 lines 5-12**).

27) . The model (Figure 7) should not appear until the Discussion, rather than at the end of the Results. Moreover, the data do not under any circumstances support the conclusion that "localization of the replication machinery is an active and regulated process that involves a gathering of replisomes replicating the Ter region independent of the chromosome linear or circular configuration".

We removed the reference to the model from the result section. We agree with the referee 1 the experiments did not support the involvement of an active and regulated process, we removed this conclusion.

28) The authors are free to say whatever they wish in the Discussion, but in the opinion of this reviewer, nearly everything in the Discussion is an unwarrented overinterpretation of the data. The authors think that they are "on to something". This is fine. Maybe so. But the current data do not lead critically to any particular model. This reviewer could make a completely different one, and could argue against that of the authors. The Discussion should focus on the more straightforward parts of the paper and the speculation should be kept to a minimum.

We are genuinely convinced that the experiments performed in this manuscript clearly demonstrate that the frequent localization of the Ter MD to mid-cell is the result of a network involving MatP, ZapB and the replication program. This is what we try to describe and to put in perspective with the observations made by other groups in the discussion and Figure 7. We modified the text of the discussion to avoid "unwarranted overinterpretation of the data" and keep the "speculation to the minimum".

Referee 2

1*) Overall, the experiments described in this paper are convincing and interesting. My only major criticisms are: (i) The paper was written in such a way that the two parts are not well connected. The MatP-ZapB portion of the paper seems very disconnected from the replication/Ter migration portion of the paper. A better connection might be achieved if the paper was reorganized to cover the topics in the order that they occur during the cell cycle.*

We have reorganized the manuscript to make it easier to read. The manuscript is now organized as follows : i) description of the localization pattern of the Ter MD in cells with circular or linear chromosome; ii) description of the involvement of replication in the migration of Ter MD to mid-cell; iii) description of the Ter MD anchoring to mid-cell through the MatP-ZapB interaction and its consequences on chromosome segregation.

2*) More effort should have been made to proofread the manuscript for clarity and grammatical errors/typos.*

We had the manuscript proofread by an american scientific colleague.

3) Although the results in Figure 3D-E suggest that ZapB is not required in the migration of the Ter region to midcell (just its anchoring there), it may be required for the movement of replisomes to midcell. This was not tested directly, and would be nice to include since it would help connect the two sections of the paper, and because of the finding that ZapB may play additional roles in nucleoid segregation on top of its interaction with MatP (see comment 7).

We did not observe any modification of the replication program in the *zapB* mutant (SSB-YPet or flow cytometry). We did not add this point to the manuscript.

4) ZapB- mutants do not display gross nucleoid defects, suggesting that either the anchoring of Ter to the septal ring is not important or that another process compensates for the lack of anchoring in ZapB- cells. This should be discussed.

We discussed this aspect in the Discussion (**page 19 lines 19-25**). As observed for a number of bacterial chromosome organization/segregation systems, the ZapB anchoring is not essential. migS, ParABS, FtsK, can be removed without massive chromosome segregation defects. Selection of a number of compensatory systems has been made ensuring correct segregation in every cell.

5) The last two sentences of the introduction, " Finally, we have shown that MatP is not responsible for the migration of the Ter MD from the pole to midcell. Instead, we present strong evidence suggesting that the capture of the Ter MD is promoted by the replisome, which is then localized at midcell, and attracts DNA", make little sense to me. Do you mean, "Instead, we present strong evidence that Ter DNA is recruited to midcell during the process of replication by midcell localized replisomes"?

We have modified the text as suggested by referee 2 (**page 5 lines 18-20**).

6) At the end of the first results section it cannot be concluded that, "FtsZ is not the only determinant controlling the process". At this point it is only clear that MatP is recruited to Z-rings. Before additional results are presented it is formally possible that a direct FtsZ-MatP interaction is all that is required for the recruitment.

The overexpression of FtsZ indicates that not all FtsZ rings are targeted by MatP. Therefore, at this step, it is already possible to propose that FtsZ is not the main target of MatP. We have modified the text to make this more explicit (**page 12 lines 15-17**).

7) Care should be taken when using the word "targeting". This implies "delivery", when in most instances I think you mean "anchoring". Also, care should be taken when using "capture" with respect to the replisome and the Ter region. In the context of this paper "capture" is very similar to "anchoring". I would instead refer to the replisome being involved in "recruiting" the Ter MD to midcell as opposed to "capturing" it.

We have modified the text all along the MS as suggested by referee 2

8) In Figure 2D, why is there a mixture of white and blue colonies on the plates that test for a 2-hybrid interaction between MatP and ZapB? Shouldn't they all be blue?

We have performed additional two hybrid experiments and presented the results with streaks on LB plates containing X-Gal and IPTG, that are uniformly blue or white ((**Figure 5D** and text from **page 13 line 22 to page 14 line 4**) . The mixture of white and blue colonies was only observed with the selection of cya+ colonies on medium containing lactose, maltose and X-Gal.

9) In figure 3 I-L a MatP- Zap+ control micrograph should be presented so the reader can compare the double mutant phenotypes to the single mutants. It is not clear why the double MatP- ZapB- strain should display an elevated nucleoid structure/segregation defect over the MatP- strain alone. MatP- mutants are presumably defective for Ter organization and anchoring to the septal ring. If the only role of ZapB is the anchoring of Ter to the septal ring (via MatP), then MatP- and MatP- ZapB- mutants should be equivalent. This suggests that ZapB has an additional role in nucleoid segregation and is mentioned in the Discussion. It would be helpful to the reader if this was acknowledged in the Results section along with "see Discussion" to indicate that this will be discussed later.

The *matP* strain is now presented on Figure 6L. We have acknowledged the fact that ZapB may have additional role on chromosome segregation in the result section (**page 16 lines 8-11**). " The synergistic effects observed with the absence of ZapA or ZapB and MatP suggest that each protein may have a yet uncharacterized function that is only revealed in the absence of its partner (see discussion)."

10) Figure 3E-F: The extra foci in the images were confusing. At first I though they represented some analysis of the foci from the outlined cell. I then realized that they are presumably ter loci in another cell adjacent to the cell the authors have highlighted. Please either crop the images to only display the cells you want to discuss in the image, or explain in the legend that the foci next to the numbers are from an adjacent cell.

As suggested by the referee 2, we have explained in the figure legend (Figure 1L and M) that the foci next to the numbers are from adjacent cells.

12) In the discussion the authors state that "bacterial two-hybrid assay revealed a direct ZapB-MatP interaction". Since the assay was performed in E. coli, it cannot be definitively ruled out that the interaction is indirect. Further in vitro work is required to determine if the interaction is direct or not.

We did not perform in vitro experiments to confirm a direct MatP-ZapB interaction. We have modified the text to point out that we can not exclude that a third partner is involved in the TerMD targeting to mid-cell (**page 18 lines 16-18**). "Because the two-hybrid assay revealing the MatP-ZapB interaction was performed in *E. coli*, we can not exclude that they do not interact directly but are brought together by a third, yet uncharacterized partner."

13) Bottom of page 7: "The system consisted of..." should read something like, "To test this, we constructed a system....."

We have modified the text as suggested by referee 2 (**page 12, lines 20-24**)

14) In figure legends it is commonly written, "...and phase contrast microscopy (grey) of the MG1655 cells grown...". It would be better to say, "...and phase contrast micrographs of MG1655 cells grown..."

We have modified the text as suggested by referee 2

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Referee 3

1) Fig. 1G: The authors overexpress ftsZ and claim that MatP frequently colocalizes with the now aberrantly localized FtsZ. The single panel shown in 1G is difficult to assess. A proper quantification of both FtsZ and MatP localization would be helpful.

We have reorganized the figure an added a quantification of the MatP /FtsZ colocalization in the FtsZ overexpression context (**Figure 4C** and text **page 12 lines 10 – 17**).

2)p. 8: The authors indicate that the plasmid containing matS sites does not localize in a matP null strain. These data, with quantification, need to be shown in the paper as they represent a critical control.

This experiment is now presented on figure S8 (text **page 13 lines 4-5**).

3)Fig. 3F: The authors show that ZapB mislocalizes in a zapA mutant, but oddly MatP doesn't colocalize anymore with ZapB. These data appear to conflict with the notion that MatP binds ZapB. The authors suggest that the ZapB foci in the zapA mutant are simply non-functional aggregates, but there's no data to substantiate such an assertion. I'm left a little uneasy about the conclusion that MatP binds ZapB for this reason and that indicated below.

Some of the authors' data supports the notion that ZapB binds MatP to retain it and the associated Ter macrodomain at mid-cell. However, this conclusion rests heavily on the finding that MatP is mislocalized in the ZapB mutant. Given that a zapB deletion causes many defects, the influence of ZapB on MatP localization could be indirect. The bacterial two-hybrid data helps support a direct relationship, but is insufficient without additional evidence. In particular, the authors need to identify a mutation in ZapB that specifically disrupts the binding of MatP in two-hybrid analysis, but does not prevent ZapB from fulfilling its other FtsZ/divisome-based roles. If such a mutant does not disrupt cell division or FtsZ/divisome ring formation, but does disrupt MatP/matS localization, I think their model will stand on firmer ground. This experiment is essential. The authors should also provide additional evidence for direct binding in the form of co-IP given how heterogeneous the colonies are in the two-hybrid analysis (which leaves me concerned that the interaction is not specific or meaningful).

To substantiate the MatP-ZapB interaction we performed additional two-hybrid assays. First, we have identified that the deletion of the 89 first amino acids of MatP dramatically increased the b-galactosidase yield in the presence of ZapB. The interaction between $MatP_{90C}$ and ZapB is readily observed on LB plates with X-Gal and IPTG. Second, we observed that the interaction between $MatP_{90C}$ and ZapB is dependent on the presence of ZapA (not the $MatP_{90C}$ -MatP interaction). Third, we have shown that the C-terminal domain of ZapB, which is not required for the interaction with ZapA (Galli et al 2011), is essential to observe the MatP-ZapB interaction (**Figure 5** and text from **page 13 line 20 to page 14 line 4**). .

4) Fig. 6B: The authors must quantify their analysis of Ter localization in the dnaBts mutant. In addition, I think it is imperative that the authors confirm these results by measuring Ter localization after perturbing DNA replication in another, independent way.

The quantification of the *dnaBts* experiment were presented in the supplementary figures, they are now presented on figure 3D-E. We have also blocked initiation of replication with a *dnaCts* allele at non permissive temperature. The results of this experiment are presented on figure 3F. In good agreement with the DnaB inactivation, the DnaC inactivation provoked a decrease in the number of cell with a centrally localized Ter focus (text from **page 10 line 25 to page 11 line 4**) .

5) The authors must reference Lau et al. (Mol. Micro., 2003) in describing the localization pattern of ter-proximal regions using MatP-mCherry localization. The work here essentially recapitulates

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previous FROS-based analyses of Ter localization in slow-growing cells published by the Sherratt group.

We agree that the data presented in the Lau et al 2003 article suggested that the Ter was frequently localized near mid-cell. This was, actually, already described in the Gordon et al 1997 and the Niki and Hiraga 1998 articles. Moreover the finding from the Lau et al article were disclaimed by the subsequent articles from the Sherratt group (Wang et al 2005 and 2006) suggesting a splitting of the Ter region to the two poles of the AB1157 cells. For all these reasons, we see limited scientific interest in referencing Lau et al 2003. We referenced the Lau et al 2003 article for the description of the FROS tags.

The authors need to address whether their findings extend beyond the MG1655 background. The relevance of the work depends on the generalizability of the results, so at least some of the key experiments should be verified in other commonly used E. coli strains.

We already described that the TerMD behave differently in a AB1157 than in a MG1655 background. The difference is only observable during an eukaryotic like cell cycle in minimal medium supplemented with glycerol for the strain AB1157 (Mercier et al 2008). As suggested by the referee 3, we performed the *matS* plasmid localization experiments (one of the key experiment of the article) in AB1157 and observed their localization to the septal ring similarly to MG1655 (supplementary Figure S8 and text **page 13 lines 2-4**)). It is also interesting to note that the two-hybrid assay was performed in the BTH101 strain which has also a different genetic background than MG1655.

2nd Editorial Decision 10 April 2012

Thank you for submtting your revised manuscript, and please excuse the delay in its re-evaluation related to the fact that not all original referees were immediately available. Referees 1 and 3 have now reviewed the paper once more and provided comments that your will find copied below. I am pleased to inform you that both referees consider the study improved and consequently now support publication without further addition of data. Referee 1 however retains some concerns regarding presentation and interpretation, which I would kindly ask you to address in a final round of minor revision. I am therefore returning the manuscript to you once more to allow you to make these text modifications. When re-uploading your final version, please also make sure to include all supplementary text and figures in a single combined PDF of sufficient quality to allow all the figures to be fully appreciated (since supplementary information will not be further copy-edited, type-set of otherwise processed by our publisher). Finally, we will also require a brief 'Conflict of Interest' statement in the manuscript text.

Once we will have received your final files incorporating these last changes, we should then be able to swiftly proceed with formal acceptance and publication of the paper!

Yours sincerely,

Editor
The EMBO Journal

REFEREE COMMENTS

Referee #1

The authors have satisfactorily answered my previous concerns. The paper is still not easy to read, particularly in the early parts. There is too much "stream of consciousness" in the presentation for my taste and I suspect that the manuscript may be too long for your standards. However, the results are very interesting and should be published without significant alteration. I would suggest to make the first part of the paper more concise and then publish. The authors are straightforward in their

presentation and analysis and there is nothing to fault in that regard. This is a very nice paper.

In the discussion, the authors suggest (p17) that bringing together of ter regions in a linear chromosome involves some "different superstructure than the chromosome itself". The authors might consider an alternative model for midcell localization of ter domains. Bates and Kleckner (2005) suggested that sister domains might push one another apart, thus bringing unreplicated regions to midcell. Such a mechanism will function regardless of whether the ter regions are linked or not, and will tend to link the process to replication, as the authors observe. By this mechanism, it would not be a "superstructure" of the chromosome which is involved but rather intrinsic features of the chromosome itself.

Referee #3

The authors have addressed most of the concerns raised by myself and other reviewers. I am still not completely convinced that MatP interacts directly with ZapB - the authors have added some additional data, but the fact that MatP doesn't colocalize with ZapB in the zapA mutant still worries me. Nevertheless, the paper as a whole has been improved and would seem appropriate for publication in EMBO.

2nd Revision - authors' response 11 April 2012

I would like to thank you and the referees for the processing of the manuscript. I believe that the manuscript has been greatly improved by your advices and the comments of the referees.

I have taken into account the last suggestions of referee 1. In the purpose to avoid “stream of consciousness “ I have removed or modified several sentences in first part of the manuscript.

Briefly:

Page 6, line 4. The following sentence has been removed:”The choreography of a MatP-GFP fusion protein is tightly controlled.”

Page 6, line 10. I have removed “to confirm” in the following sentence: “We have constructed a MatP-mCherry in order to confirm and refine this pattern of localization”

Page8, line 13. I have removed “and its possible dependence on DNA replication in the following sentence: “To analyze more precisely the timing of the Ter MD migration to mid-cell during the cell cycle and its possible dependence on DNA replication”

Page 9, line 21. I have changed “the merging of the replisome foci in one focus was not solely the consequence of two forks running in the opposite orientation on a circular track” by the merging of the replisome foci in one focus might not be solely the consequence of two forks running in the opposite orientation on a circular track”

In the discussion, page 17, I have added the following paragraph to aknowledge the fact that an alternative may be proposed for the migration of the Ter MD to mid-cell. “Alternatively, it was proposed that newly replicated sister regions might push one another apart, thus bringing unreplicated regions to mid-cell (Bates & Kleckner, 2005); such process could participate in the migration of the Ter MD to the vicinity of a mid-cell localized replication factory.”

I have added the following statement:

Conflict of interest
The authors declare that they have no conflict of interest.