molecular systems biology

A Dynamical Model of the Spindle Position Checkpoint

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(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision 06 February 2012

Thank you again for submitting your work to Molecular Systems Biology. We have now heard back from the three referees who agreed to evaluate your manuscript. As you will see from the reports below, the referees find the topic of your study of potential interest. They raise, however, a series of important concerns on your work, which, I am afraid to say, preclude its publication in its present form.

Of the concerns raised by the reviewers, the issues raised by Reviewer #1 seem to be the most fundamental. This reviewer feels that the modeling results supporting "double inhibition" (a term Reviewer #2 found confusing) could potentially be explained by an alternative mechanism where Bfa1 and Bub2 behave more like their fission yeast homologs, and feels that additional experiments may be needed to help conclusively resolve this issue.

The second point that the editor would like to emphasize is a general concern by Reviewer #2 that the novel biological insights had not been sufficiently brought to the fore in this manuscript. The editor feels that, while this work is logically and thoroughly presented, it is currently a very challenging read for scientists who are not intimately familiar with the yeast spindle position checkpoint (see also Reviewer #3's concerns regarding Fig. 6). In addition to addressing the specific points raised by each reviewer, the editor strongly encourages you to make sure that the broad importance of these findings are clearly and concisely stated, and to have the manuscript read by a non-specialist to ensure that it remains accessible to a broad biologist audience.

In addition, the editor has two additional requests related to data presentation and release:

-- Before submitting your revised work, please submit the SBML models to a public repository, such as BioModels or JWS Online, and include the accession number or confidential reviewer login in the Methods section.

-- In general we strongly encourage authors to release the underlying numeric for key experimental results, especially when these data are used to train or validate a mathematical model. To this end, we provide a new functionality that allows readers to directly download the 'source data' associated with selected figure panels (e.g. <http://tinyurl.com/365zpej>). This sort of figure-associated data may be particularly appropriate for the data presented in Fig. 2 & 3. Please see our Instructions of Authors for more details on preparation and formatting of figure source data (<http://www.nature.com/msb/authors/index.html#a3.4.3>).

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If you feel you can satisfactorily deal with these points and those listed by the referees, you may wish to submit a revised version of your manuscript. Please attach a covering letter giving details of the way in which you have handled each of the points raised by the referees. A revised manuscript will be once again subject to review and you probably understand that we can give you no guarantee at this stage that the eventual outcome will be favorable.

Sincerely,

Editor - Molecular Systems Biology
msb@embo.org

Referee reports

Reviewer #1 (Remarks to the Author):

This is an interesting paper that is addressing an important aspect of the control of cell cycle progression, the restraint of cell division when genome segregation is compromised. Specifically the manuscript addresses the Spindle Orientation Checkpoint (SPOC) of budding yeast. As outlined in the introduction the SPOC has been the subject of considerable investigation leading to an informative list of the key players and how they interact to ensure that mitotic exit is delayed when the spindle remains in the mother cell. The control of the GAP protein complex for Tem1 lies at the heart of this control because activation of this GTPase promotes mitotic exit and it is the restraint of Tem1 activation that ensures that mitotic exit does not happen until the spindle is correctly aligned. The key issue addressed by the modelling in the current study is a test of the spatial control of the interaction and activity of this GAP complex with Tem1. This is an important issue to understand. By showing how the signal is propagated throughout the cell, the results presented here provide a nice explanation for this phenomenon.

After quantitative assessment of the numbers of molecules on the SPB under different conditions, two models were tested. Both incorporate the well documented behaviour of the molecules that has been charted in the extensive cell biology that leads upto this study alongside the quantification presented in the current study. The two models differ in one respect. In the hot-spot association model, the GAP complex is only able to activate the GTPase of Tem1 on the SPB while in the Ubiquitous association model, the two proteins associate on both the SPB and the cytoplasm. Importantly the impact of Bfa1 overproduction upon the distribution of Tem1 can be used to differentiate between these two models as there will be no impact upon the residence of Tem1 at SPBs if the hot spot model is right whereas cytoplasmic Bfa1 will titrate out Tem1 away from the SPB if the ubiquitous model applies. The data clearly support the latter. Further refinement of the model comes with the introduction of the idea that the unphosphorylated Bfa1 is inactive or inhibited.

I find this to be an interesting study and the data to support the architecture of the model and the conclusions drawn. One could argue that it is important to understand the distinction between the Byr4 dependent and independent pools of Tem1 at the SPB and whether they are really equivalent in terms of MEN activation, however, I do not think that this is a major issue for the current study and does not reduce my enthusiasm for the study. I am less qualified to comment upon the mathematics in the modelling.

The major questions I found myself asking as I worked through the reasoning concerned the relationship between Bfa1 and Bub2 where my thoughts are heavily influenced by the work on the fission yeast equivalents of Bfa1/Bub2/Tem1, Byr4/Cdc16/Spg1. While there has not been an extensive characterisation of the biochemical activities of Bfa1 and Bub2 towards Tem1, the work of Furge et al. (Curr. Biol. 8: 947)has provided a very informative framework with which to view the activities of the equivalent molecules in fission yeast. Importantly Byr4 has a markedly different impact upon the activity of Spg1 depending upon whether it is partnered by Cdc16 or not. While the complex of Byr4 and Cdc16 is a very potent GAP for Spg1, Byr4 alone actually stabilizes the GTP bound state of Spg1 . Thus, in fission yeast, at least, there is no need for a GEF in the hydrolysis cycle as it can be fully supported by the intrinsic behaviour of Byr4 and Cdc16. Furthermore, Byr4 and Cdc16 show different association patterns with the SPB, indicating that there are complexes of Spg1/Byr4 that do not incorporate Cdc16 (Cerutti and Simanis J. Cell Sci. 112:2313). I therefore wonder whether the "double inhibition" phenomenon proposed here could be accounted for by a disassociation between Bub2 and Bfa1 upon dephosphorylation. If Bfa1 and Bub2 behave in the same way as Byr4 and Cdc16, then the loss of association between Bfa1 and Bub2 would flip Bfa1 into a GTP stabilising state and so assist a rapid recovery. The data presented here and before by the authors suggest that there maybe some subtle differences between Bub2 and Bfa1 recruitment as there appears to be less Bub2 on the SPBs of aligned spindles in figure 2A and that Caydasi and Pereira previously noted that "the loading of Bfa1 onto the SPB was more pronounced than that of Bub2" (Dev Cell 16;146).

In this context, and the fact that the authors indicate that Spg1 can substitute for Tem1, I wondered whether a quantitative analysis of the kinetics of Bub2.GFP association with the SPB as presented for Bfa1 in figure 2B maybe informative alongside an assessment of the association of Bfa1/Bub2 in SPOC activation and recovery? If there was a correlation between recovery and or Bfa1 phosphorylation status and Bub2 disassociation it could provide further molecular insight into a potential mechanism for the "double inhibition" model.

Beyond this consideration I had only minor comments:

1. In the model in Figure 1 Lte1 is still on the cortex when the SPB is in the daughter cell, many of the models presented for the MEN suggest that Lte1 is released at this point.

2. Please indicate in the "Species" legend what is meant by Bfa1*

3. There is no shaded area in sup Fig. 5

Reviewer #2 (Remarks to the Author):

Caydasi et al. investigate the spindle position checkpoint of budding yeast cells. They combine experiments and mathematical modeling to understand the dynamics of Tem1 activation during normal mitosis and its inhibition during spindle orientation failure. Their major finding is that Bfa1 needs to inhibit Tem1 not only at the SPB but also in the cytoplasm.

The findings are clear, the modeling is sound (although how parameters were identified is not explained) and I do not have major concerns about the results. It is a nice combination of experiment and modeling, but I do not see a major discovery out of a few extra assumptions that were needed to

fit the experimental results with the model. The major importance of these new assumptions is not convincingly presented in the manuscript, the authors rather explain the importance of some recent model supported discoveries on SAC regulation.

Specific comments:
I do not see any extra major info by the stochastic simulations, thus I would move Fig 5B and 6B to supplement. Merge Fig 5 and 6 and use the same order for the models as it is given on Fig 4D.
I found the name "double-inhibition" model a bit confusing. The same term is often used in systems biology to describe antagonistic interactions in regulatory networks. Since this is derived from the ubiquitous-association model I would propose something like "ubiquitous-inactive model" to be used instead.
In the supplement it is not properly explained what are Bfa1P4 and P5 and how these relate to the visual descriptions of the different forms of Fig 4.

The identification of parameters is explained with a sentence in the footnote of a table in the supplement. Since parameters are really important in such quantitative study, these explanations should be greatly expanded. For instance it is not clear how cytoplasmic molecular numbers were identified; these are not reported in Table 1.

The sensitivity of the models to GDP-dissociation rate was tested on Fig 6 C,D. it is clear that the double-inhibition model gives a better fit to the measured value and it works in a wider regime, but it is not clear how the other model works when it has 0% quality at the measured dissociation rate. Was that model optimized on different constrains. Again the lack of the description of parameter identification causes this confusion.

Fig 7A does not explain the best model. On the right panel it could have been added that Bfa1 could be active or inactive, depending its state.

Fig 7B can be deleted.

Reviewer #3 (Remarks to the Author):

The manuscript entitled "A dynamical model of the spindle position checkpoint" by Caydasi et al. reports on the use of in silico modeling to investigate some eatures of the spindle positioning checkpoint (SPOC) in yeast. This checkpoint ensures that cells do not activate the Mitotic Exit Network (MEN) and do not exit mitosis as long as the mitotic spindle is not properly elongate along the mother-bud axis. This manuscript focuses on the regulation of the most upstream MEN component Tem1 by its GAP complex Bub2-Bfa1. This analysis starts from the quantification of the number of molecules localizing to spindle pole bodies (SPBs), using fluorescence microscopy, to model this regulation. Indeed, according to former studies the Tem1, Bub2 and Bfa1 are thought to be active exclusively on SPBs. Both Tem1 and its GAP localize asymmetrically, mainly to the daughter bound SPB (dSPB), and a smaller fraction localizes to the mother bound SPB (mSPB). The quantification of the authors indicate that Tem1/GAP ratio is of 1:1 on the SPB in the daughter and 2:1 on the SPB in the mother. In anaphase cells with a misaligned spindle, the GAP inhibits Tem1 function. Yet the counterintuitive observation is that the same 2:1-ratio is now observed on both SPBs. The study uses computational modeling of GAP-Tem1 interactions to identify conditions whereby the GAP is able to successfully inhibit Tem1 function, despite this unfavorable ratio. The first important conclusion of the authors is that the GAP must be both active on SPB as well as in the cytosol, and that its function is to titrate Tem1 away from SPBs in cells with a misaligned spindle. After further studying reaction kinetics, the authors suggest that the large pool of unphosphorylated GAP must be inactive, which is another very interesting result.

Overall, this study makes a few very interesting points. This reviewer therefore recommends publishing this paper in Molecular Systems Biology. However, prior to publication, the following issues should be addressed by the authors.

Major points:

An important finding of the authors is that there must be a GAP-independent Tem1 pool on SPBs. Yet it would be interesting to clarify their starting observation that there seems to be more GAP-independent Tem1 on the mother SPB than in the daughter SPB. How do the authors rationalize this observation?

If Tem1 regulation takes place mainly in the cytoplasm, can the authors provide tentative explanations about why Tem1 is recruited to SPBs and what it does there? Also, why does the GAP localize mostly to one of the SPBs in cells with properly aligned spindles? What is the functional meaning of this asymmetry?

The authors went a long way to quantify the exact number of molecules present in the cell and at the poles. It seems that it would therefore useful if they would indicate for each molecule which fraction of the total population is present at each pole. It seems intuitively that this should also be a very useful information.

The text and Figures referring to the data Fig6 C,D are very confusing and should be reformulated and clarified for the non-specialists.

On page 9 paragraph 1, the authors discuss why a GAP-independent pool of Tem1 exists at SPBs. Their explanations are not easy to follow as it is written in the text. This should be made clearer.

Minor points:

-Fig1 B: Drawing depicts a different Tem1/GAP ratio than observed in Fig2 A

-Fig2 B: How was the conversion mCherry -> number of molecules performed?

-Fig2 D: Spindle elongation and mitotic exit are modified in the bub2 bfa1. Can the authors comment on this?

-Fig5 A-D: Should contain the names of the two models to make it easier to follow

1st Revision - authors' response 12 March 2012

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Title: A Dynamical Model of the Spindle Position Checkpoint

Answers to the reviewers' comments

Reviewer #1

The major questions I found myself asking as I worked through the reasoning concerned the relationship between Bfa1 and Bub2 where my thoughts are heavily influenced by the work on the fission yeast equivalents of Bfa1/Bub2/Tem1, Byr4/Cdc16/Spg1. While there has not been an extensive characterisation of the biochemical activities of Bfa1 and Bub2 towards Tem1, the work of Furge et al. (Curr. Biol. 8: 947) has provided a very informative framework with which to view the activities of the equivalent molecules in fission yeast. Importantly Byr4 has a markedly different impact upon the activity of Spg1 depending upon whether it is partnered by Cdc16 or not. While the complex of Byr4 and Cdc16 is a very potent GAP for Spg1, Byr4 alone actually stabilizes the GTP bound state of Spg1 . Thus, in fission yeast, at least, there is no need for a GEF in the hydrolysis cycle as it can be fully supported by the intrinsic behaviour of Byr4 and Cdc16. Furthermore, Byr4 and Cdc16 show different association patterns with the SPB, indicating that there are complexes of Spg1/Byr4 that do not incorporate Cdc16 (Cerutti and Simanis J. Cell Sci. 112:2313). I therefore wonder whether the "double inhibition" phenomenon proposed here could be accounted for by a disassociation between Bub2 and Bfa1 upon dephosphorylation. If Bfa1 and Bub2 behave in the same way as Byr4 and Cdc16, then the loss of association between Bfa1 and Bub2 would flip Bfa1 into a GTP stabilising state and so assist a rapid recovery.

We thank the reviewer for pointing out this interesting aspect of SIN regulation in *S. pombe*. Indeed Bfa1 has been shown to bind Tem1-GTP *in vitro* independently of Bub2. However we do not believe that this would account for a rapid activation of Tem1 for a couple of reasons. Firstly, overproduction of Bfa1 blocks mitotic exit in a Tem1-dependent manner, implying that if Bfa1 stabilizes Tem1-GTP *in vivo*, this would be inhibitory rather than permissive for MEN activation. Secondly, the interaction between Tem1 and Cdc15 was shown to be reduced in the absence of Bub2 (Ro et al., 2002), suggesting that the Bfa1-GTP-Tem1 complex would not be fully proficient in the activation of the Tem1 effector Cdc15. Thus, even if Bfa1 stabilizes Tem1 *in vivo*, this would not assist the recovery of *in vivo* active Tem1 that is required to activate the MEN. Nevertheless, to investigate whether Bub2 dissociates from Bfa1 at the SPBs, we analyzed the behavior of Bub2 and Bfa1 on single cell basis and are now showing that the SPB binding profiles of Bfa1 and Bub2 are similar (this data is now shown in Figure S3B). Therefore, we did not consider the possibility that Bub2 dissociates from Bfa1 at SPBs prior to mitotic exit in the current model. In the model, we are not considering that Bub2 dissociates from Bfa1 in the cytoplasm either because data related to the ratios of protein complexes *in vivo* would be required to make meaningful simulations.

The data presented here and before by the authors suggest that there maybe some subtle differences between Bub2 and Bfa1 recruitment as there appears to be less Bub2 on the SPBs of aligned spindles in figure 2A and that Caydasi and Pereira previously noted that "the loading of Bfa1 onto the SPB was more pronounced than that of Bub2" (Dev Cell 16;146). In

this context, and the fact that the authors indicate that Spg1 can substitute for Tem1, I wondered whether a quantitative analysis of the kinetics of Bub2.GFP association with the SPB as presented for Bfa1 in figure 2B maybe informative alongside an assessment of the association of Bfa1/Bub2 in SPOC activation and recovery? If there was a correlation between recovery and or Bfa1 phosphorylation status and Bub2 disassociation it could provide further molecular insight into a potential mechanism for the "double inhibition" model.

As discussed above, we included the time-lapse analysis of cells simultaneously carrying *BFA1*-3mCherry and *BUB2*-GFP in Figure S3B and in Results section (manuscript page 8, line 7-9). The SPB binding profile of Bub2 and Bfa1 are similar during mitosis. This observation is in line with the observation that Bfa1 and Bub2 SPB binding are interdependent (Pereira et al., 2000).

Beyond this consideration I had only minor comments:
1. In the model in Figure 1 Lte1 is still on the cortex when the SPB is in the daughter cell, many of the models presented for the MEN suggest that Lte1 is released at this point.

The release of Lte1 from the cell cortex occurs during mitotic exit and requires Cdc14 dependent dephosphorylation (Seshan et al., 2002). We are considering both cells depicted in Figure 1B to be in anaphase (prior to MEN activation), with a difference in the orientation of the spindle only. For this reason, we drew Lte1 at the daughter cell cortex in either of these cells.

*2. Please indicate in the "Species" legend what is meant by Bfa1**

We added a textual explanation of "Bfa1*" to the legend of Figure 4C.

3. There is no shaded area in sup Fig. 5

Shaded areas are now added to the Supplementary Figure 5.

Reviewer #2

The findings are clear, the modeling is sound (although how parameters were identified is not explained) and I do not have major concerns about the results. It is a nice combination of experiment and modeling, but I do not see a major discovery out of a few extra assumptions that were needed to fit the experimental results with the model. The major importance of these new assumptions is not convincingly presented in the manuscript, the authors rather explain the importance of some recent model supported discoveries on SAC regulation.

We have modified the beginning of the discussion (page 17, last paragraph) to emphasize the importance of our findings. We also shortened the discussion about SAC.

I do not see any extra major info by the stochastic simulations, thus I would move Fig 5B and 6B to supplement. Merge Fig 5 and 6 and use the same order for the models as it is given on Fig 4D.

We thank the reviewer for this suggestion. We modified the figures as suggested.

I found the name "double-inhibition" model a bit confusing. The same term is often used in systems biology to describe antagonistic interactions in regulatory networks. Since this is derived from the ubiquitous-association model I would propose something like "ubiquitous-inactive model" to be used instead.

We replaced the “double-inhibition model” with “ubiquitous-inactive model” as proposed by the Reviewer.

In the supplement it is not properly explained what are Bfa1P4 and P5 and how these relate to the visual descriptions of the different forms of Fig 4.

In the supplement, Bfa1-P4 and Bfa1-P5 denotes Bfa1 that is phosphorylated by Kin4 or Cdc5, respectively. A paragraph defining the molecular entities relevant to the models is now preceding the reaction equations (section 2 of the Supplementary Information). The relationship to Figure 4A-C should be now clearer.

The identification of parameters is explained with a sentence in the footnote of a table in the supplement. Since parameters are really important in such quantitative study, these explanations should be greatly expanded. For instance it is not clear how cytoplasmic molecular numbers were identified; these are not reported in Table 1.

A section explaining kinetics and parameterization in more detail has been added to the Materials and Methods (page 28-31, “Model Kinetics and Parameterization”). The estimation of the total cellular molecular numbers of proteins is now explained in Materials and Methods under a separate section (page 25-26, “Estimation of the total cellular molecular numbers of proteins”). Briefly, the number of protein molecules per cell was calculated by taking into account the known number of SPB bound molecules and the area sizes of measurements. The number of Bfa1 molecules per cell that was calculated by this way was similar to the one published by Ghaemmaghami et al 2003. The ratio of SPB bound protein to the whole cell protein levels are now shown in Table 1, as also suggested by Reviewer 3.

The sensitivity of the models to GDP-dissociation rate was tested on Fig 6 C,D. it is clear that the double-inhibition model gives a better fit to the measured value and it works in a wider regime, but it is not clear how the other model works when it has 0% quality at the measured dissociation rate. Was that model optimized on different constrains. Again the lack of the description of parameter identification causes this confusion.

We are sorry about this confusion. We did not optimize the models with respect to the GDP-dissociation rate coefficient because we considered the GDP-dissociation rate of Tem1 to be slightly higher than the respective rate coefficient of Spg1, the homologue of Tem1 in *S. pombe*. The choice of the parameter value is reasoned in the Supporting Information, section 1.

The quality level is a measure that combines the abilities of the model to inhibit Tem1 upon SPOC activation and to recover the level of active Tem1 upon SPOC deactivation in a nonlinear way. i.e if one of these two criteria is clearly insufficient, the quality is zero. We consider inhibition to be clearly insufficient if the amount of Tem1-GTP at the SPB remains larger than the threshold for MEN activation defined in the manuscript. Similarly, recovery is considered insufficient if Tem1-GTP at the SPB does not accumulate to a level above the same threshold. Close to the measured GDP-dissociation rates (Furge et al., 1998; Geymonat et al., 2002), the ubiquitous-association variant can inhibit Tem1, but Tem1-GTP does not recover sufficiently. Thus at these parameter values it has zero quality. The ability to recover Tem1-GTP increases with increasing GDP-dissociation rates. Consequently, at higher rates, Tem1-GTP recovery becomes sufficient and the quality rises above zero. However at even higher GDP-dissociation rates, inhibition becomes insufficient resulting in zero quality again. For clarity, the paragraph referring to figure 6 has been rewritten.

Fig 7A does not explain the best model. On the right panel it could have been added that Bfa1 could be active or inactive, depending its state.

This information is now added to the Figure 7. Active and inactive Bfa1 amounts are identical during spindle misalignment in both ubiquitous-association and ubiquitous-inactive models. Therefore, Figure 7 explains both ubiquitous-association and ubiquitous -inactive models. For clarity, we labeled the right panel with the name of both models.

Fig 7B can be deleted.

It is now deleted.

Reviewer #3

Major points:

An important finding of the authors is that there must be a GAP-independent Tem1 pool on SPBs. Yet it would be interesting to clarify their starting observation that there seems to be more GAP-independent Tem1 on the mother SPB than in the daughter SPB. How do the authors rationalize this observation?

Similar amounts of Tem1 bind to mSPB and dSPB in *bfa1*Δ *bub2*Δ cells with properly aligned spindles. This symmetric behavior has been shown before (Pereira et al., 2000) and it resembles the binding of Tem1 in cells with mis-aligned spindles in the presence of Bub2 and Bfa1. For this reason, we showed the measurements at "any SPB" in Figure 1C, and Supplementary Figure 1C rather than specifying mSPB or dSPB separately. However, we now include a graph for the comparison of the fluorescence intensities of Tem1-GFP in *bfa1*Δ *bub2*Δ cells at the mSPB and the dSPB in Figure S1D and we clarified this point in the Results section (manuscript page 8, line 17-19) to avoid any confusion.

If Tem1 regulation takes place mainly in the cytoplasm, can the authors provide tentative explanations about why Tem1 is recruited to SPBs and what it does there? Also, why does the

GAP localize mostly to one of the SPBs in cells with properly aligned spindles? What is the functional meaning of this asymmetry?

Although it is still unclear where activation of Tem1 takes place (cytoplasm or SPBs), it is clear that active Tem1 needs to bind to SPBs to initiate MEN signaling. We therefore state in various parts of the manuscript that Tem1 SPB binding is required for activation of the mitotic exit network (MEN), as shown by (Valerio-Santiago and Monje-Casas, 2011 and Gruneberg et al., 2000). Tem1 binds to the SPBs mainly via the GAP complex except for a small fraction that binds independently of it during mitosis with an increment through late anaphase. Our model suggests that if the inhibition of Tem1 by the GAP would occur exclusively at SPBs, this would not be sufficient to inactivate the entire pool of Tem1 (including the pool that binds to SPBs independently of the GAP). Therefore, it is feasible that regulation of Tem1 takes place in the cytoplasm as well as at the SPBs.

The functional importance of asymmetric SPB binding of the GAP is still not fully understood. It has been proposed that symmetric binding of Bub2 causes mitotic exit delay. However, this conclusion was based on the behavior of Bub2-9Myc, which binds to SPBs in a symmetric manner, but seems to be only partial functional (Fraschini et al., 2006). Because Cdc5 phosphorylates Bfa1 at the SPBs (Maekawa et al., 2007), we propose that the recruitment of more Bfa1 to the dSPB might be a way of increasing the inhibition of Bfa1-Bub2 GAP complex at the daughter directed SPB by Cdc5, hence allowing accumulation of active Tem1 at this SPB. This view challenges the idea that the SPB would constitute the main focus of Tem1 inhibition (Pereira et al., 2000), while supporting the observation that Bfa1-Bub2 dissociation from the SPBs is indispensable for Tem1 inhibition upon SPOC activation (Caydasi and Pereira, 2009).

The authors went a long way to quantify the exact number of molecules present in the cell and at the poles. It seems that it would therefore useful if they would indicate for each molecule which fraction of the total population is present at each pole. It seems intuitively that this should also be a very useful information.

Thank you for the suggestion. We now added this information to the Table 1.

The text and Figures referring to the data Fig6 C,D are very confusing and should be reformulated and clarified for the non-specialists.

The paragraph in the main text referring to figure 6 has been rewritten and relocated to a sub-section on its own. Additionally, the section in Materials and Methods describing the parameter scans and the associated measures has been rewritten and extended for clarity.

On page 9 paragraph 1, the authors discuss why a GAP-independent pool of Tem1 exists at SPBs. Their explanations are not easy to follow as it is written in the text. This should be made clearer.

This part has been modified to make it clearer (manuscript page 9, line 5-17).

-Fig1 B: Drawing depicts a different Tem1/GAP ratio than observed in Fig2 A

We rescaled the Tem1 and GAP in Fig1B according to Fig 2A.

-Fig2 B: How was the conversion mCherry -> number of molecules performed?

The conversion of mCherry to the number of molecules is now included in Material and Methods, page 26. Briefly, based on the fact that Bfa1-GFP and Bfa1-mCherry behaved in a similar manner (i.e. SPB binding profile during the cell cycle) and assuming that the number of Bfa1 molecules bound to SPBs should be constant independently of the tagged Bfa1 version used, we considered the number of molecules of Bfa1-3mCherry to be equal to the number of molecules of Bfa1-GFP (the molecular numbers of which was determined in comparison to Nuf2-GFP and presented in Table 1). Once the average number of proteins molecules at the SPBs were determined using the "protein-GFP / Nuf2-GFP" system (see Table 1 for molecule numbers in anaphase cells), we used these protein numbers as a reference to convert the time-lapse fluorescence intensity profiles to the number of molecules profiles. We considered that during anaphase the average fluorescence intensity of a protein-fluorophore time-lapse curve at each SPB (Figure 2B and 2D) correlates with the calculated number of molecules (Table 1) of the same protein at the corresponding SPBs.

-Fig2 D: Spindle elongation and mitotic exit are modified in the ∆bub2∆bfa1. Can the authors comment on this?

This is an interesting point. The timing of mitotic exit (calculated according to the Mob1 localization) occurs on average 2 min earlier in *bfa1*Δ *bub2*Δ cells than in WT cells. However considering the 1 min time resolution of the time-lapses, we avoid making comments on this difference, as more detailed analysis (with better time resolution) would be necessary to draw sound conclusions.

-Fig5 A-D: Should contain the names of the two models to make it easier to follow

The names were included.

Acceptance letter 30 March 2012

Thank you again for sending us your revised manuscript. We are now satisfied with the modifications made and I am pleased to inform you that your paper has been accepted for publication.

Before, we can send this work to production we have a few minor format and content issues that we ask you to address:

1. Thank you for submitting your model to the BioModels repository. In general, we also ask authors to include model files as supplementary material, to ensure quick and easy access for readers. Could you please provide a single zip file containing the model files? The Table of Contents in the Supplementary Materials PDF should then be modified to include this supplementary file (listing it as "separate file" instead of a page number).

2. The text and line-art in the current Figure images is somewhat blocky, which becomes very noticeable when zooming in. You will receive a higher-quality final product if you remake these figures in a professional-quality vector graphics program like Illustrator or the free alternative Inkscape, and save the figures directly as EPS or PDF files. This is a minor point, and the current images are probably acceptable, if you would rather not remake the figures.

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Sincerely,

Editor - Molecular Systems Biology

msb@embo.org

Reviewer #1 (Remarks to the Author):

The authors have addressed all the points raised in my first round of reviewing and I am happy to support publication of this interesting manuscript.

Reviewer #2 (Remarks to the Author):

The authors followed all my suggestions and considered the comments of the other referees as well. I do not have further comments.

