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The E2F functional analog SBF recruits the Rpd3(L) HDAC, via Whi5 and Stb1, and the FACT chromatin reorganizer, to yeast G1 cyclin promoters

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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 12 July 2009

Thank you for submitting your manuscript for consideration by The EMBO Journal. It has now been seen by two referees whose comments to the authors are shown below. A third referee who agreed to review the manuscript for us has not been able to return his/her report as quickly as initially expected and he/she still has not sent us his/her report. As the other two reports are in fair agreement I am taking a preliminary decision on your manuscript now, based on the two enclosed reports, in order to save you from unnecessary loss of time. This decision is still subject to change should the third referee offer strong and convincing reasons for doing so.

As you will see referees 1 and 3 are positive about the manuscript and would support publication here after appropriate revision. We would therefore be able to consider a revised manuscript if you can address the referees' criticisms in an adequate manner along the lines suggested. I would like to add that - as also referee 3 has pointed out to the editor - the wording "E2F paralogue" for SBF in the title should be replaced by "functionally analogous protein" as E2F and SBF are (structurally) unrelated proteins.

I would suggest at this point to start revising the paper along the lines suggested by the reviewers, and to also include satisfactory answers to any criticisms that might be raised in the third report, which we will forward to you as soon as we receive it.

I should remind you that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS

Referee #1 (Remarks to the Author):

These investigators have assayed CLN1 and CLN2 promoter occupancy by ChIP analysis across the cell cycle. These two genes are transcribed in late G1 and their regulation bears striking resemblance to that of E2F/Rb targets in metazoans. They have assayed the transient binding profiles of gene-specific activators and repressors (Swi4, Swi6, Whi5 and Stb1). They have also studied the recruitment of chromatin modifiers and the disassembly of nucleosomes. This study follows a previous Mol Cell manuscript, which describes another Swi4/Swi6 regulated gene HO. In that case, they assayed binding at three different locations in the HO promoter and found that chromatin modification and disassembly was mechanistically different at each location. This new study is quite parallel in strategy and of similarly high technical quality, and it reports a similarly complex order of events. The principle findings are that Swi6 recruits FACT to late G1 promoters, which is important for nucleosome eviction and transcription. This is consistent with what was found at HO, but unlike HO, Asf1 is not required. They also find that Rpd3L and cyclin-dependent kinase are recruited and this recruitment requires Swi6 and either Whi5 or Stb1. Unfortunately, there is not a simple relationship between CLN1, 2 transcript levels and FACT, Swi6, Whi5 or Stb1 activity, so there are clearly some missing puzzle pieces. Nevertheless, their data is of high quality, they have a high fold-enrichment and smooth curves in their ChIPs across the cell cycle. They have expanded their scope to explore other late G1 transcripts and there is no doubt that this work provides an important foundation for other studies of these two very important promoters. I think the manuscript would be more valuable if some of the following suggestions could be addressed.

1. The introduction could be more informative as to what these proteins do. The description of Stb1 is especially confusing because it is once described as an inhibitor and then as an activator. References are provided, but there is no explanation of these apparent contradictions.
2. They use the fact that Whi5 specifically interacts with Swi4 (and not Mbp1) to explain some of their results and they reference two companion papers. However, this is an unresolved issue. Only one of the two papers cited comes to that conclusion. The second paper offers positive results that Whi5 interacts with both Swi4/Swi6 complexes and Mbp1/Swi6 complexes. Glossing over this does no service to their readers. Perhaps they could do some experiments to offer some further support to their preferred interpretation and help clarify the matter.
3. RME1 is postulated to explain the residual expression of CLN1,2. Here again, testing that speculation directly might clarify matters and move the field forward.

As more of these kinds of studies are carried out, it is clear that the mechanism of chromatin disassembly and transcriptional activation varies widely. These investigators do a very capable job and add a substantial amount of new information about some of the critical components that participate in the transient activation of these promoters. However, there is a great deal of complexity that cannot be explained, and some of these proteins, like Stb1, seem more mysterious than ever.

Referee #3 (Remarks to the Author):

In this manuscript, the authors investigate the mechanism of transcriptional activation by the SBF complex of budding yeast. They provide compelling evidence for a mechanism in which SBF recruits first repressors, then activators of G1-specific transcription to promoters of CLN1, CLN2 and other important cell cycle-regulatory genes. The switch between repression and activation depends on activity of the cyclin-dependent kinase (CDK), Cdc28, which is shown to interact genetically with regulators of G1 transcription and physically with chromatin of genes activated in

G1. This work strengthens the analogy between G1 control in metazoan and fungal systems, with SBF acting similarly to the E2F transcriptional activator, and the SBF-associated repressor Whi5 playing the role of the retinoblastoma tumor suppressor protein Rb in mammalian cells. Although these functions (and the analogy with E2F and Rb) had been suggested previously, the actual mechanism is novel (and also reminiscent of the mechanisms proposed for Rb/E2F function). It involves the recruitment and eviction of the repressive histone deacetylase (HDAC) Rpd3(L) complex, and the reciprocal exclusion and subsequent recruitment of the chromatin-remodeling FACT complex, during the repressed and activated phases of transcription, respectively. The experiments are well done, the results are mostly clear-cut and the interpretations are reasonable. I have one substantive concern or question, and some minor comments and criticisms, but overall, this is a solid and important contribution to the understanding of cell-cycle and transcriptional regulation.

My major concern:

1. I am surprised by the modest-to-negligible effect of simple whi5 deletion on RNA levels of CLN1 and CLN2, given the role of Whi5 as a repressor, and also given that whi5 deletion boosts RNA levels 3-4-fold in a stb1 whi5 double mutant relative to the stb1 single deletion (Fig. 2F). The authors invoke "additional mechanisms...to limit" G1 cyclin mRNA expression (p. 6), but I wonder whether a positive effect might have been masked by looking only in asynchronously grown cells. They miss a chance to investigate this further, by looking at RNA levels in synchronized populations of WT and stb1 whi5 cells, which they do analyze for FACT recruitment by ChIP (Fig. 5). In these experiments, there is clear enhancement of FACT-binding to HO, CLN1 and CLN2, with essentially normal cell-cycle timing, which makes the lack of an effect on transcript levels all the more puzzling.

Minor or cosmetic concerns:

1. There is a typo in the title ("EF2" instead of E2F).
2. I have some problems with the organization and preparation of figures. First (and most important), I think the temporal profiles of RNA accumulation in synchronized cells are too central to be relegated to a supplemental figure (S4, which, oddly, is cited before any other supplemental figure in the text). I would suggest either incorporating at least some of these data into Fig. 1, or including them as a free-standing figure in the manuscript proper. Second (and also quite significant), the data in Fig. 4, which are absolutely essential for understanding the proposed mechanism, are presented in a most confusing way. It took me several rounds of looking at the legend of symbols at the bottom and then at the panels, before I was able to decipher what was represented by each plot. Even then, I did so by ignoring the symbols themselves (which were too small to make out on either my screen or the hard copy). Once I "got it," it was all quite logical, so some simple re-packaging of these data (with bigger symbols!) would easily spare readers the disorientation I experienced.
3. I would suggest adding a figure (or at least a panel) showing the model for transcriptional regulation by SBF in cartoon form. This paper is adding significant new detail to previous papers on the subject, and those not already in the know will be greatly helped by a schematic diagram bringing HDACs and histone chaperone/chromatin remodeling complexes into the picture.
4. On p. 5, a passing mention is made of Rpd3(L) and Rpd3(S) complexes, but no explanation of the functional distinction between them is given. Again, to rationalize the focus here on Rpd3(L) to the non-afficionado (or to those who may have read something about Rpd3(S)), a one-liner explaining that Rpd3(L) is found at promoters and thought to repress transcription, whereas Rpd3(S) recruitment occurs in actively transcribed regions as part of a chromatin maintenance pathway, would be helpful.
5. On p. 9, last paragraph, the call-out to Fig. 4C, D, is incorrect, as the preceding sentence refers (exclusively, as far as I can tell) to previously published results.
6. On p. 15 of Discussion, to explain the surprisingly mild effects of whi5 mutations on Rpd3(L) recruitment, the authors raise the possibility that "Cdc28 also acts on Stb1," which is quite reasonable considering published evidence that Stb1 is a CDK substrate in vitro and in vivo-papers that should be cited here in support of the authors' suggestion.

Additional Correspondence 27 July 2009

We have now received the third report on your manuscript (see below). As you will see also referee 2 is supportive about publication of the paper here in principle. Still, he/she feels that major revisions will be required before the paper will be publishable here. I would therefore like to ask you to address this referee's criticisms together with the issues brought up by the other two referees (that I had forwarded to you in my initial decision letter) in a revised manuscript.

Again, please be reminded that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORT

Referee #2 (Remarks to the Author):

The manuscript "The EF2 paralog SBF recruits Rpd3(L) HDAC and FACT chromatin complexes to yeast G1 cyclin promoters" by Takahata et al describes an analysis of the role of chromatin remodeling factors in the regulation of SBF and MBF regulated gene expression. The authors show that both Rpd3(L) and FACT complex components bind to SBF and MBF promoters prior to or at the time the genes are expressed and that their recruitment to SBF promoters depends upon SBF. Furthermore, both Whi5 and Stb1 transcriptional regulators contribute to, and are required for, the recruitment of Rpd3(L) to SBF-regulated promoters. The same factors are also required for the recruitment of the Cdc28 CDK. They demonstrate that FACT is required for a transient nucleosome eviction occurring at the time of expression at both SBF and MBF promoters. Finally, disruption of STB1 and WHI5 can suppress mutations in FACT and Cdc28 consistent with a role of those factors in overcoming chromatin repression at G1 cyclin promoters.

This work presents a number of interesting and novel observations regarding the role of Rpd3(L) and FACT complex in regulation of G1 genes. It extends and generalizes the author's prior analysis of the role of chromatin remodeling factors at the HO promoter, a complex promoter regulated by SBF and other factors. Importantly, it establishes a role for the FACT complex in nucleosome remodeling at SBF and MBF-regulated promoters and implicates transcriptional regulators in its recruitment. The experiments are generally well conceived and executed. However, the relationship between the transcriptional regulators of SBF and MBF is confusing and there are a number of other shortcomings that will need to be addressed for some of the experiments to be clearly interpreted. The manuscript would profit from additional care in the presentation and interpretation of results. The specifics of those criticisms are presented below.

Specific points:

1. There are two significant typos in the title of the paper. "EF2" should be "E2F". "FACT chromatin complexes" should be "FACT chromatin remodeling complexes", or something similar.

2. Analysis of transcript level during the cell cycle should be presented for experiments where the interpretation involves the relationship between factor binding and transcriptional activity. It is surprising that transcript analysis is presented only in the last supplementary figure. This is particularly important for the experiments presented in figures 2 (see below) and 5. In figure 5, it is unclear how transcription correlates with the double peak of FACT binding to CLN promoters. This seems critical for the interpretation of that data.

3. There is disagreement between the Ho et al and Costanzo et al papers concerning the role of Stb1 at promoters, the former suggesting it is an inhibitor and the later that it participates in repression but also in activation as it is required for maximal expression of G1 genes. Most of the data presented in this paper seems to show in that mutation of STB1 reduces gene expression (figs 2 & s2). The genetic interactions with spt16-11 presented in figure 6 also appear to support a positive role for Stb1. The interaction between whi5 and stb1 is also not adequately addressed. It is difficult to relate the effect ability of whi5 to suppress the transcriptional defect in stb1 mutants while enhancing its effect on both Rpd3 and FACT binding. Because prior analysis suggests that there are different effects depending on the phase of the cycle, the effect of stb1, whi5 and the double mutant on gene expression in synchronized cells is necessary adequately represent its effect on gene expression.

4. The overall trend in figure 4 appears consistent with the author's conclusions. However, it is surprising that the data is so noisy while the error for independent data points is low. The source of the error bars should be described. Are these from multiple independent experiments or do they just represent the error in sample replicates? This should be clarified throughout the manuscript.

5. There is an error in the legend provided within figure 4. "RNA ChIP in swi6" should read "RNA in swi6".

6. Whi5 is not observed at native MBF-regulated promoters and does not affect the expression of MBF-dependent genes (de Bruin et al, 2004). This is difficult to reconcile with an effect of whi5 mutation on Cdc28 binding to those promoters (fig. S3) and the lack of an effect of stb1 whi5 mutant on FACT binding to SBF targets in figure 5. This may be a consequence of evaluating binding in asynchronous cells. This should be addressed. In addition, the statement on pages 11 and 14, that Whi5 interacts specifically with Swi4, is inaccurate. It is probably best to say that Swi4 is required for the interaction of Whi5 with SBF.

7. Both stb1 and whi5 mutations appear to suppress the growth defect of cdc28-13. Although this is consistent with a putative role for Cdc28 in regulating transcription, it is equally likely that it is a consequence of the effect of those mutations on the expression of G1 cyclins, which activate Cdc28 for other non-transcriptional functions required for growth. Consequently, it is difficult to draw clear conclusions from those genetic interactions.

8. The authors state in the abstract that Swi6 recruits FACT, which seems to imply a direct interaction. However, they have only shown that Swi6 is required for recruitment.

9. The paper would profit greatly from a careful consolidated description of the authors overall interpretation of the roles of Rpd3(L) and FACT in the regulation of G1 genes and the relative contribution of each to the regulation of SBF and MBF by Stb1 and Whi5.

1st Revision - Authors' Response 31July 2009

The E2F functional analog SBF recruits the Rpd3(L) HDAC and the FACT chromatin reorganizer to yeast G1 cyclin promoters

Reviewer #1.

Specific comments:

1. The introduction could be more informative as to what these proteins do. The description of Stb1 is especially confusing because it is once described as an inhibitor and then as an activator. References are provided, but there is no explanation of these apparent contradictions.
The introduction has been modified providing more background on both Stb1 and Whi5.

2. They use the fact that Whi5 specifically interacts with Swi4 (and not Mbp1) to explain some of their results and they reference two companion papers. However, this is an unresolved issue. Only one of the two papers cited comes to that conclusion. The second paper offers positive results that Whi5 interacts with both Swi4/Swi6 complexes and Mbp1/Swi6 complexes. Glossing over this

does no service to their readers. Perhaps they could do some experiments to offer some further support to their preferred interpretation and help clarify the matter.
The revised paper contains a co-immunoprecipitation experiment (Fig 3) showing that Whi5 binds to SBF and not to MBF.

3. RME1 is postulated to explain the residual expression of CLN1,2. Here again, testing that speculation directly might clarify matters and move the field forward.
The revised paper contains experiments (Fig S6) examining CLN2 expression in rme1 pob3 and rme1 swi6 double mutant strains. The double mutants have additive defects in CLN2 transcription.

Reviewer #2.

Specific points:

1. There are two significant typos in the title of the paper. "EF2" should be "E2F". "FACT chromatin complexes" should be "FACT chromatin remodeling complexes", or something similar.
The title of the manuscript has been revised.

2. Analysis of transcript level during the cell cycle should be presented for experiments where the interpretation involves the relationship between factor binding and transcriptional activity. It is surprising that transcript analysis is presented only in the last supplementary figure. This is particularly important for the experiments presented in figures 2 (see below) and 5. In figure 5, it is unclear how transcription correlates with the double peak of FACT binding to CLN promoters. This seems critical for the interpretation of that data.
In the revised paper we present CLN2 transcript data early, as part of Fig 1A.
The reviewer asks how transcription correlates with the double peak of FACT binding to CLN promoters. This is shown in Fig 4E and 4F, where gene transcription is analyzed in the same time course as FACT binding. We do not know the meaning of the double peak but in the Discussion we speculate that the two waves of FACT binding could contribute to nucleosome disassembly and reassembly, respectively.

3. There is disagreement between the Ho et al and Costanzo et al papers concerning the role of Stb1 at promoters, the former suggesting it is an inhibitor and the later that it participates in repression but also in activation as it is required for maximal expression of G1 genes. Most of the data presented in this paper seems to show in that mutation of STB1 reduces gene expression (figs 2 & s2). The genetic interactions with spt16-11 presented in figure 6 also appear to support a positive role for Stb1. The interaction between whi5 and stb1 is also not adequately addressed. It is difficult to relate the effect ability of whi5 to suppress the transcriptional defect in stb1 mutants while enhancing its effect on both Rpd3 and FACT binding. Because prior analysis suggests that there are different effects depending on the phase of the cycle, the effect of stb1, whi5 and the double mutant on gene expression in synchronized cells is necessary adequately represent its effect on gene expression.
The revised manuscript includes an experiment (Fig 2E) examining CLN1 and CLN2 RNA levels in synchronized wild type and stb1 whi5 double mutant cells.

4. The overall trend in figure 4 appears consistent with the author's conclusions. However, it is surprising that the data is so noisy while the error for independent data points is low. The source of the error bars should be described. Are these from multiple independent experiments or do they just represent the error in sample replicates? This should be clarified throughout the manuscript.
Error bars in ChIP assays and in RT-qPCR assays reflect the standard deviation of three replicate PCRs. This is stated in the Materials And Methods section.

5. There is an error in the legend provided within figure 4. "RNA ChIP in swi6" should read "RNA in swi6".
This has been fixed.

6. Whi5 is not observed at native MBF-regulated promoters and does not affect the expression of MBF-dependent genes (de Bruin et al, 2004). This is difficult to reconcile with an effect of whi5 mutation on Cdc28 binding to those promoters (fig. S3) and the lack of an effect of stb1 whi5 mutant

on FACT binding to SBF targets in figure 5. This may be a consequence of evaluating binding in asynchronous cells. This should be addressed. In addition, the statement on pages 11 and 14, that Whi5 interacts specifically with Swi4, is inaccurate. It is probably best to say that Swi4 is required for the interaction of Whi5 with SBF.
The reviewer is correct that it is unexpected that a whi5 mutation would affect Cdc28 binding to MBF-dependent genes. We have included a sentence pointing out that this could be an indirect effect of the whi5 mutation in these asynchronous cells.
The text on (previous) pages 11 and 14 concerning Whi5 interaction with Swi4 have been re-written in the context of our new co-immunoprecipitation experiment.

7. Both stb1 and whi5 mutations appear to suppress the growth defect of cdc28-13. Although this is consistent with a putative role for Cdc28 in regulating transcription, it is equally likely that it is a consequence of the effect of those mutations on the expression of G1 cyclins, which activate Cdc28 for other non-transcriptional functions required for growth. Consequently, it is difficult to draw clear conclusions from those genetic interactions.
We agree that one cannot draw clear conclusions from these experiments. The language we use is suitably cautious, "**suggesting** that FACT and Cdc28 play an important role in activation of G1 targets genes."

8. The authors state in the abstract that Swi6 recruits FACT, which seems to imply a direct interaction. However, they have only shown that Swi6 is required for recruitment.
The revised manuscript includes an experiment (Fig 3C) showing an interaction between purified Swi6 and FACT, demonstrating a direct interaction.

9. The paper would profit greatly from a careful consolidated description of the authors overall interpretation of the roles of Rpd3(L) and FACT in the regulation of G1 genes and the relative contribution of each to the regulation of SBF and MBF by Stb1 and Whi5.
We have modified the discussion, and included a new figure with a model (Fig 7).

Reviewer #3.

My major concern:

1. I am surprised by the modest-to-negligible effect of simple whi5 deletion on RNA levels of CLN1 and CLN2, given the role of Whi5 as a repressor, and also given that whi5 deletion boosts RNA levels 3-4-fold in a stb1 whi5 double mutant relative to the stb1 single deletion (Fig. 2F). The authors invoke "additional mechanisms...to limit" G1 cyclin mRNA expression (p. 6), but I wonder whether a positive effect might have been masked by looking only in asynchronously grown cells. They miss a chance to investigate this further, by looking at RNA levels in synchronized populations of WT and stb1 whi5 cells, which they do analyze for FACT recruitment by ChIP (Fig. 5). In these experiments, there is clear enhancement of FACT-binding to HO, CLN1 and CLN2, with essentially normal cell-cycle timing, which makes the lack of an effect on transcript levels all the more puzzling.
We were also surprised that the whi5 mutation only modestly affects CLN RNA levels. The revised manuscript includes an experiment (Fig 2E) examining CLN1 and CLN2 RNA levels in synchronized wild type and stb1 whi5 double mutant cells. This experiment shows a slightly larger increase in CLN RNA in the stb1 whi5 double mutant cells, but the effect is still modest.

Minor or cosmetic concerns:

1. There is a typo in the title ("EF2" instead of E2F).
The title of the manuscript has been revised.

2. I have some problems with the organization and preparation of figures. First (and most important), I think the temporal profiles of RNA accumulation in synchronized cells are too central to be relegated to a supplemental figure (S4, which, oddly, is cited before any other supplemental figure in the text). I would suggest either incorporating at least some of these data into Fig. 1, or including them as a free-standing figure in the manuscript proper. Second (and also quite significant), the data in Fig. 4, which are absolutely essential for understanding the proposed

mechanism, are presented in a most confusing way. It took me several rounds of looking at the legend of symbols at the bottom and then at the panels, before I was able to decipher what was represented by each plot. Even then, I did so by ignoring the symbols themselves (which were too small to make out on either my screen or the hard copy). Once I "got it," it was all quite logical, so some simple re-packaging of these data (with bigger symbols!) would easily spare readers the disorientation I experienced.
In the revised paper we present CLN2 transcript data as part of Fig 1A.
We have reorganized the data in Fig 4, retaining the data for the wild type strains here and moving the data for the swi6 mutant into a supplemental figure. This allows us to make the graphs larger and to label them more clearly.

3. I would suggest adding a figure (or at least a panel) showing the model for transcriptional regulation by SBF in cartoon form. This paper is adding significant new detail to previous papers on the subject, and those not already in the know will be greatly helped by a schematic diagram bringing HDACs and histone chaperone/chromatin remodeling complexes into the picture.
The revised manuscript contains a summary cartoon figure (Fig 7).

4. On p. 5, a passing mention is made of Rpd3(L) and Rpd3(S) complexes, but no explanation of the functional distinction between them is given. Again, to rationalize the focus here on Rpd3(L) to the non-afficionado (or to those who may have read something about Rpd3(S)), a one-liner explaining that Rpd3(L) is found at promoters and thought to repress transcription, whereas Rpd3(S) recruitment occurs in actively transcribed regions as part of a chromatin maintenance pathway, would be helpful.
A sentence has been added to the introduction.

5. On p. 9, last paragraph, the call-out to Fig. 4C, D, is incorrect, as the preceding sentence refers (exclusively, as far as I can tell) to previously published results.
This sentence has been re-written.

6. On p. 15 of Discussion, to explain the surprisingly mild effects of whi5 mutations on Rpd3(L) recruitment, the authors raise the possibility that "Cdc28 also acts on Stb1," which is quite reasonable considering published evidence that Stb1 is a CDK substrate in vitro and in vivo-papers that should be cited here in support of the authors' suggestion.
This section has been re-written, and the appropriate references have been cited.

2nd Editorial Decision 12 August 2009

Thank you for sending us your revised manuscript. Our original referee 2 has now seen it again, and you will be pleased to learn that in his/her view you have addressed the referees' criticisms in a satisfactory manner, and that the paper will therefore be publishable in The EMBO Journal.

Before this will happen, however, I was wondering whether you would like to consider addressing the minor issues suggested by referee 2 (see below). Please let us have a suitably amended manuscript as soon as possible. I will then formally accept the manuscript.

Yours sincerely,

Editor
The EMBO Journal

REFEREE REPORTS

Referee #2 (Remarks to the Author):

The revised manuscript by Takahata et al is a greatly improved manuscript that addresses all of the substantive concerns of the previous round of review. Although a number of issues remain

confusing, there are a number of clear conclusions drawn from the data and even the data regarding the confusion issues is clear and worthy of publication. However, there are several residual issues that I think are worthy of comment. In the interest of greater clarity or accuracy, the authors may want to address these issues in the context of the manuscript prior to publication.

1. The title makes no mention of Stb1 and Whi5, which are central elements of this study. Would it make sense to refer to regulators of SBF rather than just SBF in the title?

2. Figure 3A is a confirmation of published findings of de Bruin et al, 2004 and consequently may be more appropriately published in the supplement and referred to in the text.

3. It seems odd that the behavior of FACT at promoters regulated by MBF is similar to that at HO, which is regulated by SBF. The behavior at CLN1 and CLN2 is different. This seems worthy of comment in the text.

4. The behavior of whi5 mutants is a point of confusion on the part of the authors and another reviewer. It is important to realize that Whi5 functions primarily in cells in which the "sizer" is functioning (see de Talia et al; Skotheim et al). The consequence of deletion is that SBF regulated genes are activated prematurely in those cells. In a mixed population of mothers and daughters this effect is difficult to observe. In addition, cells that have been synchronized by a method that allows growth of the bud prior to birth will mask the effect of the whi5 mutation on gene expression. This likely explains the absence of a strong effect in this population. A short comment on this issue would probably be valuable to readers.

5. The last sentence of the first paragraph on page 11 is confusing.

6. One of the uses of "while" should be eliminated in the first sentence of the first complete paragraph on page 8.

2nd Revision - Authors' Response 12 August 2009

Response to reviewer's comments

1. The title makes no mention of Stb1 and Whi5, which are central elements of this study. Would it make sense to refer to regulators of SBF rather than just SBF in the title?
The Title has been revised to "The E2F functional analog SBF recruits the Rpd3(L) HDAC, via Whi5 and Stb1, and the FACT chromatin reorganizer, to yeast G1 cyclin promoters."

2. Figure 3A is a confirmation of published findings of de Bruin et al, 2004 and consequently may be more appropriately published in the supplement and referred to in the text.
This figure has been moved to the supplement.

3. It seems odd that the behavior of FACT at promoters regulated by MBF is similar to that at HO, which is regulated by SBF. The behavior at CLN1 and CLN2 is different. This seems worthy of comment in the text.
We have changed the text as follows.

Paragraph 4 of discussion ends like this:
Finally we see two peaks of FACT binding at *CLN1* and *CLN2*, one at 25 min after release and one at 50 min. The two waves of FACT binding could contribute to nucleosome disassembly and reassembly, respectively, but further studies are needed to verify this speculation. Overall, this underscores the way that the same factors can play fundamentally different roles at different promoters.

Changed to:
Finally we see two peaks of FACT binding at *CLN1* and *CLN2*, one at 25 min after release and one at 50 min. The two waves of FACT binding could contribute to nucleosome disassembly and

reassembly, respectively, but further studies are needed to verify this speculation. It is also surprising that the kinetics of FACT binding to *HO* most closely resemble that of *CDC21*, a MBF-activated gene, instead of *CLN1* and *CLN2*. Although *HO*, *CLN1* and *CLN2* are all SBF-dependent genes, there are important differences, including stringent chromatin repression uniquely seen at the *HO* promoter (S.T., Y.Y, and D.J.S., manuscript in preparation). Overall, these results underscore the way that the same factors can play fundamentally different roles at different promoters.

4. The behavior of whi5 mutants is a point of confusion on the part of the authors and another reviewer. It is important to realize that Whi5 functions primarily in cells in which the "sizer" is functioning (see de Talia et al; Skotheim et al). The consequence of deletion is that SBF regulated genes are activated prematurely in those cells. In a mixed population of mothers and daughters this effect is difficult to observe. In addition, cells that have been synchronized by a method that allows growth of the bud prior to birth will mask the effect of the whi5 mutation on gene expression. This likely explains the absence of a strong effect in this population. A short comment on this issue would probably be valuable to readers.
We have changed the text as follows.

Paragraph 3 of discussion starts with:
stb1 and *whi5* mutations have different effects on transcriptional activation of cyclin genes (*Fig 2D*). RNA levels are decreased in a *stb1* mutant, consistent with Stb1 functioning as a transcriptional activator (de Bruin et al, 2008). However, a *whi5* mutation did not result in the increased expression expected when this negative regulator of SBF-dependent genes is removed (Costanzo et al, 2004; de Bruin et al, 2004). Surprisingly, a *whi5* mutation suppresses the defect in SBF-dependent gene expression caused by *stb1*.

Changed to:
stb1 and *whi5* mutations have different effects on transcriptional activation of cyclin genes (*Fig 2D*). RNA levels are decreased in a *stb1* mutant, consistent with Stb1 functioning as a transcriptional activator (de Bruin et al, 2008). Interestingly, a *whi5* mutation suppresses the defect in SBF-dependent gene expression caused by *stb1*. One might expect to see increased expression of G1 cyclin genes in the *whi5* single mutant, as *WHI5* is a negative regulator of SBF-dependent genes (Costanzo et al, 2004; de Bruin et al, 2004). However, the major effect of a *whi5* mutation is seen in assays measuring the size of cells when they pass START (Di Talia et al, 2007). Although SBF-dependent genes are activated prematurely in a *whi5* mutant, this would not be seen in our experiments where the synchrony method allows growth of the bud prior to cell division.

5. The last sentence of the first paragraph on page 11 is confusing.
We have changed the text as follows.

Old sentence:
However, unlike the situation at the *HO* promoter, these events eventually occur at *CLN2*, so FACT and Swi6 do not have an absolute requirement.

New sentences:
This is unlike the situation at the *HO* promoter where the gene is completely transcriptionally inactive in FACT or SBF mutants. In contrast, nucleosome eviction, coactivator recruitment, and gene expression eventually occur at *CLN2* in these mutants, and thus FACT and Swi6 are not absolutely required here.

6. One of the uses of "while" should be eliminated in the first sentence of the first complete paragraph on page 8.
We have changed the text as follows.

Old sentence:
While Stb1 interacts with the Swi6 subunit present in both SBF and MBF (de Bruin et al, 2008), while there is discrepancy in the literature as to whether Whi5 interacts with both SBF and MBF or with SBF alone SBF (Costanzo et al, 2004; de Bruin et al, 2004).

New sentence:
While Stb1 interacts with the Swi6 subunit present in both SBF and MBF (de Bruin et al, 2008), there is discrepancy in the literature as to whether Whi5 interacts with both SBF and MBF or with SBF alone SBF (Costanzo et al, 2004; de Bruin et al, 2004).

