Distinct role for Mediator tail module in regulation of SAGA-dependent, TATA-containing genes in yeast

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Transaction Report:

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

1st Editorial Decision 26 May 2011

Thank you for submitting your manuscript for consideration by The EMBO Journal. I apologise for the length of time it has take to have your manuscript reviewed, and since the final referee is not responded to multiple emails I have decided to make a decision on the manuscript based on the two reports that I have. As you will see form the comments, despite both referees finding the role of the Mediator tail domain in regulated SAGA-dependent genes interesting they currently provide mixed recommendations. Overall, several aspects of the study need to be improved both experimentally and with the presentation. In addition the role of the tail domain in regulating SAGA dependent genes needs to be further extended to support a general mechanism. These issues are central to the main conclusions of the paper and need to be satisfactorily addressed before the manuscript can be further considered, it should be noted that both referees must be satisfied with the revised manuscript for it to be further considered for The EMBO Journal. Nevertheless, given the interest in the study, should you be able to address the concerns we would be happy to consider a revised version of the manuscript for publication in The EMBO Journal.

I should remind you that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. When you submit a revised version to the EMBO Journal, please make sure you upload a letter of response to the referees' comments. Please note that when preparing your letter of response to the referees' comments 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 initiative, please visit our website: http://www.nature.com/emboj/about/process.html
Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Editor
The EMBO Journal

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REFEREE COMMENTS

Referee #1

The Mediator complex plays a central role in the transcription of eukaryotic genomes since mutations that affect some of its subunits lead to complete cessation of transcription by RNA polymerase II. It is constituted of four modules, one of which, the tail interacts with the transcription activators. It is thought that this interaction brings the Mediator on promoters then stimulates preinitiation complex formation and ultimately the recruitment of Pol II.

In this manuscript, Morse and colleagues report on their work about the characterization of the tail module in yeast. One of the unsolved question about the tail is why are all its subunits non essential and whether or not all the signals from the activators are transmitted through interactions with the Mediator tail. It was observed that the Med15/Gal11 and Med3 subunits of the Mediator play similar redundant roles since single deletion affect the transcription of similar genes and since the double deletion increased the transcription defect of that same set of genes. The effect of the double tail subunit mutation on recruitment of preinitiation complex components is investigated. Interestingly, tail subunit mutations differentially affect the recruitment of TBP and possibly Pol II depending on the gene considered (see below). The authors then use a "compendium" approach developed by the Kupiec laboratory to look if other mutations might affect a set of genes that resembles the set affected by the tail subunits double deletion. Using an argument based on the similarity of phenotypes of SAGA and Mediator tail mutations, it is suggested that the tail module might be responsible for the regulation of the SAGA-dependent genes as defined by the Pugh laboratory. I am not very familiar with this approach here but would probably use the correlation only as an argument to look further. Accordingly, the authors investigate PIC assembly at SAGA-dependent or TFIID-dependent promoters and find that it is affected at the former but not the later in the double med15 med3 deletion mutant.

While the conclusion that the tail module is required for the regulation of SAGA-dependent genes are potentially interesting, there are a number of problems both minor and more important with this manuscript. In particular, the quality of some of the data is not to the level expected in EMBO Journal. Moreover, a number of results are over interpreted. The text is quite long and could probably be shortened. In its present form the manuscript is thus not suitable for publication in this journal. The problems are detailed hereafter.

Comments about the Figures:

-The Northern blots on Figure 1 are of poor quality. The amounts of RNA loaded in each lane in panels A, C and D vary widely. Because of this, it is impossible for the reader to know if the conclusions drawn by the authors are warranted or not.

-On Figure 1B, the med2 deletion mutant still allows the recruitment of Med15 upon CHA1 induction while med3 does not. This situation is completely unexpected because previous work by Myers et al. indicated that a deletion mutant of Med2 lost Med3 and Med15. It is thus expected that med2 deletion phenotype should include all the phenotype of med3 med15 double deletion mutant. The authors should have an explanation for this.

-The authors conclude from the experiment on Figure 1E that med15 med3 loses the entire tail module when they did not look at the Mediator itself but at the occupancy of the subunits by ChIP. One could admit that chipping can be used as a proxy but the authors have not looked at
Med16/Sin4. They should thus talk about a tail submodule constituted of Med2 Med3 and Med15.

-Figure 2C Northern blot is fraught with the same problems as those on Figure 1.

-In Figure 2D, some mRNA fold changes are expressed relative to the wild-type while other are normalized to the med15 med3 deletion. This is inconsistent.

-The Figure 2B is redundant with Figure 3. Both are about the genome-wide effect of med15 med3 double mutation plus other Mediator mutants in Figure 3. One experiment is performed on an Affymetrix platform while the second uses an Agilent based platform. The strains were grown in different media. I am not sure that the strains used are exactly the same, which might explain while in Figure 2B there are a large number of differences between the med15-myc med3 and med15 med3 double deletion, which is not apparent on Figure 3. Moreover, strain LS10 had a duplication of chromosome V (the one which is used as negative control in ChIP experiment... See the comments for Figure 4). The data that relate to the expression of genes mapping to this chromosome were removed. Similarly the wild-type used for Figure 3 had an aneuploidy of chromosome II and the expression analysis of its genes was not performed. I wondered if this is legitimate because one could argue that aneuploidies could have indirect effects on the expression of genes on other chromosomes. The Holstege laboratory has probably a good grasp of this problem, which should be discussed in the Results section instead of being buried in the Materials and Methods. In fact, Figure 3 would suffice to make the point that the double mutations affect the same set of genes as the single mutations, only more strongly. It would also be enough for the GO analysis. I should add that an analysis, not as thorough though, of the single mutants has already been published in van de Peppel et al, Molecular Cell (2005). I think that the first two Figures could be condensed.

-Figure 4 investigates the occupancy of Med18/Srb5, TBP and Pol II on a number of genes and shows that the double deletion mutant affects it differentially depending on which gene one looks at. The presentation of the data is curious because a log scale is used. It prevents easy reading of the Figure and compresses the error bars. Moreover, it seems that some of the genes, which are actively transcribed, might have less Pol II or TBP than a negative control. The negative control is indicated as chromosome V in the figure legend. You have to go to the Materials and Methods to see that it is a non-transcribed portion of chromosome V. Which portion is not stated. Bear in mind that in some strains there is a duplication of chromosome V. I would like to be sure that it is not the case for the experiments presented in this Figure.

-Figure 4D is not used and should be removed.

-Figure 5A and B have no scales.

-Interpretation and presentation of Figure 6 might be correct but the use of the log scale prevents the reader to assess the data. For example, looking at the figure one does not have the impression (except for PHO84) that there is an important difference in the effect of med15 med3 double mutant on Pol II occupancy neither on SAGA-dependent nor on TFIID-dependent genes. The difference is quite obvious tough for Med18 and TBP.

Comments about the text:

-The use of the gene nomenclature is not consistent throughout the text and Figures. Some subunit genes have several names that are used more or less randomly. The research community on Mediator has agreed on a standard nomenclature (Bourbon et al. Molecular Cell, 2004). It should be used throughout.

-Page 7 line before the last: It is stated that Mediator tail subunits function redundantly. There are four subunits but just one pair, Med15 Med3, has been examined. The authors should not extend their observation on just one pair of subunits to the whole set.

-Page 8 to 11: The authors discuss at length the effect of med15 med3 double mutation and
mutations of the tail and Cyclin-Cdk module. Some of this analysis has already been performed by van de Peppel et al. (2005). Maybe this part should be reduced and the results discussed with an emphasis on what is new. In this section, one finds again the notion that tails subunits function redundantly when actually this has been demonstrated for a single pair. At the end of this section, the author state that the med15 med3 double mutation affect a larger number of genes compared to the single mutant. This does not show in Figure 3. Why?

-Throughout the Results section the authors use liberally the adjective "many" (see for example page 22 lines 8, 10, 11). To be able to make my own opinion, I would like to have figures, in the form of percentages for example, instead.

-The title of the section at the end of page 17 goes "Loss of the Mediator tail module...". It should be changed to reflect that only med15, med3 and possibly med2 are lost. Med 16 might still be there.

-Page 20 second paragraph, I do not see why Med15 and Med3 qualify as two of the three "principal" tail module subunit. Med16 tail subunit controls another set of gene. Its role is as important as that of Med15 or Med3.

Referee #3

This is an interesting paper identifying a cohort of genes whose expression is strongly dependent on the Mediator tail domain, and suggesting that Mediator is recruited to SAGA-dependent genes by a tail-dependent mechanism whereas TFIID-dependent genes recruit Mediator independently of the tail domain. By analyzing the CHA1 gene they realized that elimination of Gal11 and Med3 simultaneously was required to reduce expression of this gene and went on to conduct genome-wide expression analysis of mutants lacking single tail subunits versus both Gal11 and Med3. In this way, they identified a group of ~160 genes that behaved similarly in showing greater reductions in the double mutant versus the single mutants. Interestingly, these genes are significantly enriched in genes shown previously to be dependent on SAGA and SWI/SNF versus those more strongly dependent on TFIID versus SAGA for efficient transcription. ChIP analysis of 5-6 genes of each class revealed that recruitment of Mediator, TBP and PolII was substantially reduced in all of the SAGA-dependent examples whereas the TFIID-dependent genes showed only modest reductions in Mediator, TBP and PolII occupancy in the gal11,med3 double mutant. They also presented ChIP results indicating that the genes they examined which display increased, rather than decreased expression in the gal11,med3 double mutant display insignificant levels of Mediator in WT cells that do not increase in the mutant, leading them to propose an indirect mechanism for the derepression of these genes in cells lacking both Mediator tail subunits.

Critique: This paper is significant in revealing an unexpected difference between SAGA- vs. TFIID-dependent genes in the mechanism of Mediator recruitment, wherein the TFIID-dependent class seems not to require the tail subdomain for significant Mediator recruitment and presumably relies on Head or Middle domain subunits for the major component of Mediator recruitment. These results fit with previous findings indicating that the tail domain is a target for recruitment by inducible activator proteins, which tend to regulate SAGA-dependent promoters; however, it was not anticipated that the tail subdomain would be largely dispensable for Mediator recruitment at housekeeping genes. The most important concern I have with this paper is whether they have analyzed a sufficient number of SAGA versus TFIID dependent promoters (only 5-6 of each) to make a valid general conclusion about the differing requirements for Mediator recruitment by these large sets of hundreds or thousands of genes, and also whether any "cherry picking" occurred for the 11 genes they have presented in Fig. 6. A ChIP-chip or ChIP-Seq genome-wide analysis of Mediator occupancy in WT versus the gal11, med3 double mutant would provide the most thorough test of their hypothesis. Short of this, which I view as being outside the scope of this study, the authors could be asked to extend their ChIP analysis to another 5-10 genes of each class and indicate clearly whether they are reporting the results of every gene they analyzed. In particular, it would be good to examine target genes of several well-studied activators (Gal4, Gcn4, Adr1), which tend to regulate SAGA-dependent genes, under inducing conditions. (It's possible that they could also find and cite results from the literature on a few such inducible target genes, where tail subunits were shown to be required for robust Mediator recruitment by ChIP.) There are a few other issues that require attention as well, described below.
Specific criticisms:

- Unfortunately, the Northern analysis relies on rRNA as the loading control, which is not necessarily a good indicator of the yield of mRNA population in an RNA sample, as the latter is much more susceptible to RNase contamination. In some cases, they have also done quantitative RT-PCR and included mRNA internal controls in these measurements. The latter approach should be extended to confirm every key conclusion that currently rests on Northern analysis alone, or they should probe the Northern blots with an internal mRNA control.

- p. 9, lines 10-11, p. 10, lines 12-16, and middle of p. 21. These observations all beg the question of whether the upregulation of these genes occurs via a tail-dependent antagonism of the negative effect of the Cyc-Cdk module on transcription. If so, then eliminating GAL11 and MED3 in srb8 or srb10 mutants would produce no derepression in addition to that seen in the srb single mutant. Alternatively, it could be that the Cyc-Cdk module just happens to exert a repressive effect on a large proportion of SAGA-dependent genes in a manner that is not functionally linked to the role of the tail in activation, in which case the gal11,med3 mutations would not show epistasis with the srb mutations. Considering the number of times the authors raise this issue, they should consider analyzing the gal11, med3, srb10 triple mutant in an effort to distinguish between these different models.

- p. 13-14: They argue that the derepression of genes observed in the gal11,med3 mutant does not involve a direct role of the Mediator tail in repression because they cannot detect Mediator at most of these genes in WT cells. However, considering that the srb4-ts affects expression of nearly all Pol II genes, it seems possible that Mediator occurs at these genes at functionally significant levels that are below the detection limit of their ChIP experiments. In addition, eliminating tail subunits could inactivate their hypothetical role in repression without leading to a detectable increase in Mediator occupancy. Hence, they might want to be more cautious in concluding an indirect role in repression based on ChIP data alone. If repression is indirect, how do they envision it might occur? Is reduced competition for GTFs and PolII with SAGA-dependent promoters a possibility to mention?

- p. 17, lines 3-5: This sentence seems to contradict their prior conclusion that inactivating Gal11,Med3 derepresses genes by an indirect mechanism.

- p. 18, lines 3-7: Regarding the 11 SAGA- or TFIID-dependent genes chosen for ChIP analysis in Fig. 6, one would like to know how they all behaved in the microarray analysis, i.e. was expression of the SAGA-dependent genes reduced while that of the TFIID-dependent genes unchanged or elevated in the gal11,med3 mutant?

- p. 23, top. I'm not sure it's wise to refer to the tail module as a "sensor", which generally implies a function in detecting levels of metabolites inside or outside the cell. It seems more likely that the tail is required to support the functions of activators that are responsive to metabolite levels, and thus is a component of signal-transduction mechanisms that couple metabolite levels to changes in transcription, but is not actually a sensor. (They actually espouse this view later on at the bottom of this page.)

- p. 25: I believe there is evidence for Gal4 and Gcn4 of a contribution of Mediator head subunits, in addition to the tail domain, in recruitment of Mediator. Perhaps there is a continuum in which SAGA-dependent activators are at the "tail" end of the spectrum, while TFIID-dependent activators are at the "head" end of the spectrum for importance among Mediator subunits in recruitment by activators.
Point-by-point response to referees’ critiques:

Referee #1:

**Major comments:** There are a number of problems both minor and more important with this manuscript. In particular, the quality of some of the data is not to the level expected in EMBO Journal. Moreover, a number of results are over interpreted. The text is quite long and could probably be shortened . . . The problems are detailed hereafter.

Because of the general nature of this criticism, it is most appropriate to respond to the detailed comments:

Comments about the Figures: -The Northern blots on Figure 1 are of poor quality. The amounts of RNA loaded in each lane in panels A, C and D vary widely. Because of this, it is impossible for the reader to know if the conclusions drawn by the authors are warranted or not.

We have replaced Fig. 1A with the former Supplementary Figure 1 displaying qPCR data corresponding to the Northern blot that was originally in Fig. 1A. We have also replaced Figs. 1C and D with newly generated RT-qPCR data.

-On Figure 1B, the med2 deletion mutant still allows the recruitment of Med15 upon CHA1 induction while med3 does not. This situation is completely unexpected because previous work by Myers et al. indicated that a deletion mutant of Med2 lost Med3 and Med15. It is thus expected that med2 deletion phenotype should include all the phenotype of med3 med15 double deletion mutant. The authors should have an explanation for this.

I believe this comment must refer to Myers et al. (1999) PNAS 96: 67. This paper reports that deletion of Med2 results in loss of Pgd1 (Med3) and vice versa from purified Mediator complex. Med15/Gal11 was not assessed in that paper because of its co-migration with Rpb2 on a protein gel. Zhang et al. (2000) MCB 24: 6871 discuss earlier reports, including Myers et al. (1999) that suggest loss of multiple tail module subunits occurs in single subunit deletion mutants, and they suggest that this loss may occur only upon biochemical fractionation of Mediator away from other proteins. Zhang et al. also find comparable levels of Mediator from whole cell extracts prepared from single subunit deletion mutants and from wild type yeast binding to GST-Gcn4, while binding is severely reduced in double mutants (e.g. gal11Δ med2Δ). They also point out that distinct in vivo phenotypes are associated with gal11Δ, med2Δ, and pgd1Δ (med3Δ) yeast, making it unlikely that this triad of subunits is lost in any single deletion mutants. This apparent discrepancy was noted previously in our manuscript (p. 11), and we have now added text to indicate that it may result from different effects manifesting in vivo than seen in biochemical fractionations.

It is also important to recognize, as stated in the manuscript, that the gal11-myc allele behaves as a partial loss of function mutant in med3Δ yeast, as also reported by Zhang et al.

-The authors conclude from the experiment on Figure 1E that med15 med3 loses the entire tail module when they did not look at the Mediator itself but at the occupancy of the subunits by ChIP. One could admit that chipping can be used as a proxy but the authors have not looked at Med16/Sin4. They should thus talk about a tail submodule constituted of Med2 Med3 and Med15.

This is a fair point, and we have amended the text accordingly.

-Figure 2C Northern blot is fraught with the same problems as those on Figure 1.

We believe the Northern blot in Fig. 2C has value in showing in a visually dramatic fashion the increased expression seen for some genes in the tail module double mutants, and the differences between induction of MET32 and SPS19 seen in gal11Δ med3Δ or gal11-myc med3Δ
yeast as compared to srb8Δ or srb10Δ mutants. Figure 2D already showed supporting quantitative data for the Mediator tail module double mutants, and we have now added qPCR data also for the srb8Δ and srb10Δ strains.

-In Figure 2D, some mRNA fold changes are expressed relative to the wild-type while other are normalized to the med15 med3 deletion. This is inconsistent.

We apologize for this inconsistency and have corrected the figure.

-The Figure 2B is redundant with Figure 3. Both are about the genome-wide effect of med15 med3 double mutation plus other Mediator mutants in Figure 3. One experiment is performed on an Affymetrix platform while the second uses an Agilent based platform. The strains were grown in different media. I am not sure that the strains used are exactly the same, which might explain while in Figure 2B there are a large number of differences between the med15-myc med3Δ and medi5 med3Δ double deletion, which is not apparent on Figure 3. Moreover, the concordance dependently in each lab), values do not depend on the relative scale used and comparison of results from the two labs is possible. Furthermore, the concordance in expression, as noted by the referee, also testifies to the consistency of the data. We also agree that the aneuploidies indicated (which evidently occurred during growth for at least one of the three biological replicates used for the indicated experiments) could also potentially be of concern.

It is true that there is a degree of redundancy between data in Figs. 2 and 3. However, we nevertheless prefer to keep Figures 2 and 3 separate. First, Figure 2 documents the similarity in altered gene expression between gal11Δ med3Δ and gal11-myc med3Δ strains, and includes data to show increased expression for some genes in these mutants which is sometimes also seen for the cyclin-CDK module subunit deletions (srb8Δ and srb10Δ) and sometimes not; the data in Figure 3 is entirely used to compare gene expression in single subunit and double subunit deletions from the Mediator tail module, and also srb8Δ and srb10Δ strains. Second, the data for Figure 2 was obtained from yeast grown in YPD, while the data in Figure 3 derives from yeast grown in CSM (as indicated in the figure legends). We feel that the distinct emphasis for the two figures, and the value of corroborative data for yeast grown under different growth conditions, outweighs the small savings in journal space that would result from condensation of these figures (e.g. by removing the data for yeast grown in YPD). In addition, it was necessary to use data from yeast grown in YPD to compare with other genome-wide expression data from the Chromatin Modifier Compendium from Steinfeld et al. (Nature Genetics (2007) 39: 303), while comparison with the data for single subunit deletion mutants (Fig. 3), which was obtained from yeast grown in CSM (data from the Holstege lab), required the double mutants also to be grown in CSM. With regard to strains, the same double mutants were used in Figs. 2 and 3, and throughout the manuscript. Probably the reason it may appear at first glance that there are more differences between the gal11-myc med3Δ and the gal11Δ med3Δ strains in Fig. 2B than in Fig. 3 is that many more genes are included in the latter, so a much smaller fraction show this difference in Fig. 3 than in 2B. This is because Fig. 2B only includes genes having changes in expression of at least 1.5 fold for gal11-myc med3Δ or gal11Δ med3Δ yeast (grown in YPD), while Fig. 3 includes genes having changes in expression of at least 1.5 fold for any of the eight mutants shown (grown in CSM). This is indicated in the figure legends.

It is correct that the array platforms used to measure expression were different for the double mutants (done using Affymetrix arrays, in the Morse lab) than for the single mutants (done using custom arrays in the Holstege lab). (Note: the data for single mutants shown here derives from experiments done subsequent to the van de Peppel 2005 manuscript.) Because all values used in the expression analysis are relative to wild type yeast analyzed on the same platform (i.e., the wild type control was performed independently in each lab), values do not depend on the relative scales used and comparison of results from the two labs is possible. Furthermore, the concordance in expression, as noted by the referee, also testifies to the consistency of the data. We also agree that the aneuploidies indicated (which evidently occurred during growth for at least one of the three biological replicates used for the indicated experiments) could also potentially be of concern.
particularly because of potential indirect effects. However, it is also true that these aneuploidies affect only a minor fraction of yeast genes, and the data obtained ignoring the affected chromosomes are sufficiently robust to support the broad results reported here, namely the increased effect of double mutants compared to single mutants in the Mediator tail module, and the preferred role for the Mediator tail module in regulating SAGA-dependent, TATA-containing genes rather than TFIID-regulated genes. These results were also corroborated in separate experiments examining individual loci. We also note that such aneuploidies are not infrequently observed in microarray experiments (Hughes et al. (2000) Nature Genetics 25:333), and are difficult to detect outside of conducting an array experiment.

I think that the first two Figures could be condensed.

It was not clear to us whether this referred to Figs. 2 and 3, or 1 and 2, or how specifically the figures might be condensed. As noted above, we prefer to retain the information in Figs. 2 and 3, as we believe they are useful to the reader. We also believe that all of the data in Fig. 1 is needed to support the conclusions drawn in the manuscript.

-Figure 4 investigates the occupancy of Med18/Srb5, TBP and Pol II on a number of genes and shows that the double deletion mutant affects it differentially depending on which gene one looks at. The presentation of the data is curious because a log scale is used. It prevents easy reading of the Figure and compresses the error bars. Moreover, it seems that some of the genes, which are actively transcribed, might have less Pol II or TBP than a negative control. The negative control is indicated as chromosome V in the figure legend. You have to go to the Materials and Methods to see that it is a non-transcribed portion of chromosome V. Which portion is not stated. Bear in mind that in some strains there is a duplication of chromosome V. I would like to be sure that it is not the case for the experiments presented in this Figure.

In our view, the log scale is the most appropriate measure to use for presentation of expression data and for occupancy data from ChIP experiments. Because the data derives from Ct values obtained from real time PCR, the log values best correspond to the actual experimental measurements. This also means that error measurements derive from these values (which correspond to log values, not to fold change); hence, error bars are not artificially compressed by this usage. Furthermore, conversion of the (measured) log values to fold change results in a large asymmetry, as upregulation covers the scale from 1 to infinity whereas downregulated values only extend from 0 (well, slightly more than zero) to one. This is discussed, among other sources, in Knudsen, “A Biologist’s Guide to Analysis of DNA Microarray Data” (2002) Wiley-Liss. Other labs also use log scale for ChIP data (e.g. Venters et al. (2011) Mol. Cell 41: 480; Linder et al. Biochem. Biophys. Res. Comm. (2006) 349:948; Zhu et al., Mol. Cell (2006) 22: 169), and most reports of nucleosome occupancy use a log scale for presentation. Some of these reports, like ours, sometimes find lower values for individual loci than for “background” measurements.

We apologize for omitting the details of our use of a non-transcribed region of Chromosome V as control. We now include a citation for the use of this control, which has been used by a number of labs for ChIP experiments in yeast. Since the errors for our ChIP measurements are fairly small, and the aneuploidy for Chromosome V observed in the microarray data occurred during growth in YPD and was not present in the parent strain (e.g. it was not found in the microarray data for the same strain grown in CSM for Figure 3), we are confident that the ChIP data reflects data from non-aneuploid yeast. Furthermore, use of other loci (e.g. SNR6) instead of the non-transcribed Chromosome V region for normalization did not noticeably alter overall results.

-Figure 4D is not used and should be removed.

We apologize for this error and have removed Figure 4D.

-Figure 5A and B have no scales.

They do; the y axes are vertical from the “0” value on the x-axis.

-Interpretation and presentation of Figure 6 might be correct but the use of the log scale prevents the reader to assess the data. For example, looking at the figure one does not have the impression (except for PHO84) that there is an important difference in the effect of med15 med3 double mutant
on Pol II occupancy neither on SAGA-dependent nor on TFIIID-dependent genes. The difference is quite obvious tough for Med18 and TBP.

We have already discussed our preference for the log scale, but to help the reader we have included in the manuscript text the average fold changes seen for the eleven genes assayed for each category. We have also included in the text describing the results of Fig. 6 p-values that indicate that the trends observed of less effect on ChIP of Srb5/Med18, TBP, and Pol II for TFIIID-dependent genes than for SAGA-dependent genes, although not always conspicuous when comparing individual genes, are significant.

Comments about the text: -The use of the gene nomenclature is not consistent throughout the text and Figures. Some subunit genes have several names that are used more or less randomly. The research community on Mediator has agreed on a standard nomenclature (Bourbon et al. Molecular Cell, 2004). It should be used throughout.

There are two reasons that we don’t want to dispense with the old, non-standardized nomenclature for Mediator subunits: 1) it makes it more difficult to connect with older literature, including the use of search terms based on that literature (e.g. “Srb5”), and 2) the old names carry information that is helpful as a mnemonic device for information about their properties (e.g.mutations in the tail module subunit GAL11 result in defects in activation of genes by the activator Gal4, and Srb subunits in the head module were recovered as repressors of mutations in subunits of RNA polymerase B (pol II)). In fact some of the signatories on the Bourbon et al. paper (Hinnebusch, Winston) continue to stick with the old nomenclature in their published work. Our compromise, which we hope is acceptable (and which others have also adopted), is to use both old and new names when they are different (Gal11/Med15), although sometimes in figures it is cumbersome to do so for space reasons. We have carefully gone through the manuscript to ensure that we consistently adopt this compromise.

Page 7 line before the last: It is stated that Mediator tail subunits function redundantly. There are four subunits but just one pair, Med15 Med3, has been examined. The authors should not extend their observation on just one pair of subunits to the whole set.

We agree, and have amended the text accordingly.

Page 8 to 11: The authors discuss at length the effect of med15 med3 double mutation and mutations of the tail and Cyclin-Cdk module. Some of this analysis has already been performed by van de Peppel et al. (2005). Maybe this part should be reduced and the results discussed with an emphasis on what is new. In this section, one finds again the notion that tails subunits function redundantly when actually this has been demonstrated for a single pair. At the end of this section, the author state that the med15 med3 double mutation affect a larger number of genes compared to the single mutant. This does not show in Figure 3. Why?

We have carefully read through the pertinent section of the manuscript to ensure that we do not present discussion that is redundant with previous work of van de Peppel et al. Regarding the statement that the med15 med3 double mutation affects a larger number of genes compared to the single mutants, this can be seen by noting that many of the genes in Clusters 2 and 5 in Figure 3 that show change in the double mutant are essentially unaffected (show little blue or yellow color) in the med3 or med15 single mutants. In the revised manuscript, we have modified this statement to “double mutants show greater effects” to include both genes showing stronger effects in the double mutant than in single mutant, as well as those showing little or no effect in the single mutant but affected in the double mutant. We also have added Fig. 3B and Supplemental Fig. S1B to show graphically the increased effect on transcription, and increased number of genes showing an effect for a given cutoff value (e.g. down by 2 fold) in the gal11/med15∆ med3∆ mutant compared to either single mutant, or to med3Δ.

Throughout the Results section the authors use liberally the adjective "many" (see for example page 22 lines 8, 10, 11). To be able to make my own opinion, I would like to have figures, in the form of percentages for example, instead.
We have altered text to replace “many” with percentages or other less vague indicators for most of the places it was used.

-The title of the section at the end of page 17 goes "Loss of the Mediat or tail module...". It should be changed to reflect that only med15, med3 and possibly med2 are lost. Med 16 might still be there.

We have revised this subtitle to “Disruption of tail module function preferentially affects PIC assembly on SAGA-dependent promoters”.

-Page 20 second paragraph, I do not see why Med15 and Med3 qualify as two of the three "principal" tail module subunit. Med16 tail subunit controls another set of gene. Its role is as important as that of Med15 or Med3.

We apologize for this careless phrasing, which we have now corrected.

Referee #2:

Major comments: The most important concern I have with this paper is whether they have analyzed a sufficient number of SAGA versus TFIID dependent promoters (only 5-6 of each) to make a valid general conclusion about the differing requirements for Mediator recruitment by these large sets of hundreds or thousands of genes, and also whether any "cherry picking" occurred for the 11 genes they have presented in Fig. 6. A ChIP-chip or ChIP-Seq genome-wide analysis of Mediator occupancy in WT versus the gal11, med3 double mutant would provide the most thorough test of their hypothesis. Short of this, which I view as being outside the scope of this study, the authors could be asked to extend their ChIP analysis to another 5-10 genes of each class and indicate clearly whether they are reporting the results of every gene they analyzed. In particular, it would be good to examine target genes of several well-studied activators (Gal4, Gcn4, Adr1), which tend to regulate SAGA-dependent genes, under inducing conditions. (It's possible that they could also find and cite results from the literature on a few such inducible target genes, where tail subunits were shown to be required for robust Mediator recruitment by ChIP.)

The promoters examined in the original version of this manuscript were indeed all that we had examined at that point (i.e., they were not cherry-picked to support a hypothesis). We have extended this analysis to examine eleven promoters from each class (and show all of those we have examined in Fig. 6), and we provide p-values to support the hypothesis that stronger effects are seen on recruitment of Srb5/Med18, TBP, and Pol II in gal11/med15Δ med3Δ yeast for SAGA-dependent genes than for TFIID-dependent genes. We also cite, in the Discussion of these results, studies from the Hinnebusch and Kuras labs showing that Mediator, TBP, and Pol II recruitment are reduced in mutants of the Gal11/Med2/Med3 submodule.

Other specific criticisms:

-Unfortunately, the Northern analysis relies on rRNA as the loading control, which is not necessarily a good indicator of the yield of mRNA population in an RNA sample, as the latter is much more susceptible to RNase contamination.

As noted in the response to Referee #1, we have now included quantitative RT-PCR data to support all conclusions previously relying on Northern analyses.

-p.9, lines 10-11, p. 10, lines 12-16, and middle of p. 21. These observations all beg the question of whether the upregulation of these genes occurs via a tail-dependent antagonism of the negative effect of the Cyc-Cdk module on transcription. If so, then eliminating GAL11 and MED3 in srb8 or srb10 mutants would produce no derepression in addition to that seen in the srb single mutant. Alternatively, it could be that the Cyc-Cdk module just happens to exert a repressive effect on a large proportion of SAGA-dependent genes in a manner that is not functionally linked to the role of the tail in activation, in which case the gal11,med3 mutations would not show epistasis with the srb mutations. Considering the number of times the authors raise this issue, they should consider analyzing the gal11, med3, srb10 triple mutant in an effort to distinguish between these different models.
An epistasis experiment similar to that indicated (comparison of med2Δ, cdk8Δ, and med2Δ cdk8Δ on genome-wide expression) was reported by van de Peppel et al. (2005); hence, our citing of that previous work in the text indicated by the referee. To clarify this we have added text stating that the work was supported by epistasis analysis.

- **p. 13-14**: They argue that the derepression of genes observed in the gal11,med3 mutant does not involve a direct role of the Mediator tail in repression because they cannot detect Mediator at most of these genes in WT cells. However, considering that the srb4-ts affects expression of nearly all Pol II genes, it seems possible that Mediator occurs at these genes at functionally significant levels that are below the detection limit of their ChIP experiments. In addition, eliminating tail subunits could inactivate their hypothetical role in repression without leading to a detectable increase in Mediator occupancy. Hence, they might want to be more cautious in concluding an indirect role in repression based on ChIP data alone. If repression is indirect, how do they envision it might occur? Is reduced competition for GTFs and PolII with SAGA-dependent promoters a possibility to mention?

This is a fair point, and we have added an appropriate proviso to the indicated text (p. 14). A number of indirect effects could be postulated, including that preferred by the reviewer; in the absence of stronger evidence, we would prefer not to speculate on this in the manuscript.

- **p. 17 , lines 3-5**: This sentence seems to contradict their prior conclusion that inactivating Gal11,Med3 derepresses genes by an indirect mechanism.

Mediator could collaborate with repressors by either direct or indirect means (e.g. regulating the level of the repressor or of a cofactor needed for repressor function, etc.).

- **p. 18, lines 3-7**: Regarding the 11 SAGA- or TFIID-dependent genes chosen for ChIP analysis in Fig. 6, one would like to know how they all behaved in the microarray analysis, i.e. was expression of the SAGA-dependent genes reduced while that of the TFIID-dependent genes unchanged or elevated in the gal11,med3 mutant?

We have added this information to the text. More of the SAGA-dependent genes than of the TFIID-dependent genes examined show decreased transcription in the mutant, reflecting the enrichment seen in the expression analysis.

- **p. 23, top.** I'm not sure it's wise to refer to the tail module as a "sensor", which generally implies a function in detecting levels of metabolites inside or outside the cell. It seems more likely that the tail is required to support the functions of activators that are responsive to metabolite levels, and thus is a component of signal-transduction mechanisms that couple metabolite levels to changes in transcription, but is not actually a sensor. (They actually espouse this view later on at the bottom of this page.)

We agree and have modified the heading accordingly.

- **p. 25**: I believe there is evidence for Gal4 and Gcn4 of a contribution of Mediator head subunits, in addition to the tail domain, in recruitment of Mediator. Perhaps there is a continuum in which SAGA-dependent activators are at the "tail" end of the spectrum, while TFIID-dependent activators are at the "head" end of the spectrum for importance among Mediator subunits in recruitment by activators.

Indeed, a role for the tail module in regulating targets of Gal4 and Gcn4 does not preclude other subunits from having important roles as well; these may or may not be redundant with tail module function. In the interests of not extending what is already a moderately long manuscript, we would prefer to leave this interesting possibility to the reader’s devices. However, we have added a sentence (p. 20, top) to explicitly indicate that the TFIID/SAGA dichotomy is not absolute.
Thank you for submitting your revised manuscript for consideration by the EMBO Journal. I apologise once again for the length of time it has taken to reach a decision but the manuscript, it seems to have fallen victim to certain circumstances on both occasions, firstly an unresponsive referee and then the holiday season. As you will see from the comments below both referees find the study has been significantly strengthened and recommend publication once a number of minor text changes have been implemented.

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

Thank you for the opportunity to consider your work for publication.

Yours sincerely,
Editor
The EMBO Journal

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REFEREE COMMENTS

Referee #1

The revised version of the manuscript by Ansari et al. has taken my comments into account. New experiments have been performed. It is now clearer and more precise. It provides significant new insights into the mechanism of the tail module of Mediator in transcription activation. I support its publication in the EMBO Journal.

There are still some minor typos and problems that concern the nomenclature in particular and that should be corrected:

- p4, five lines before the end: "of the the tail module" should be replaced by "of the tail module".
- p6: There is a reference to a PhD thesis (He, 2008). I could not find it easily using Google. It would, if possible, better to give a reference to a peer-reviewed journal.
- p8, line 4: "yeast grow" should read "yeast grows".
- Throughout the manuscript, the figure legends and in Figure 5A-B the "gal11 med3 yeast" is sometimes spelled as "gal11 med3 yeast" without space. This typo should be modified.
- p24, eight lines before the end: Replace "Med14" by "Rgr1/Med14".
- p37: To be coherent med16/sin4 should be replaced by sin4/med16; med15/gal11 by gal11/med15; "analyzed by by qRT-PCR" by "analyzed by qRT-PCR"; the phrase concerning the ethidium bromide staining which a reminiscence of the previous version should be removed; Gal11-myc med3delta mixes protein and genetic nomenclature and should be changed to something coherent as for example what is written on the second line of p38.
- p38, five lines before the end: CDK8 should be written Cdk8.
- p39: The three occurrences of Srb5 should be replaced with Srb5/Med18.
- Figure 4D: The label Cdcl2-ind should be replaced by CdCl2-ind.
- Supplemental data, line 4: of "(gal115delta..." should be corrected.

Referee #3

I am satisfied with the authors' analysis of additional promoters in fig. 6, and with their responses to my other specific criticisms.