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The structure of an Iws1/Spt6 complex reveals an interaction domain conserved in TFIIS, Elongin A and Med26

Marie-Laure Diebold, Michael Koch, Erin Loeliger, Vincent Cura, Fred Winston, Jean Cavarelli and Christophe Romier

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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 06 October 2010

Thank you for submitting your manuscript for consideration at The EMBO Journal. I have now received the final report from the two referees that have evaluated your study, which I enclose below. As you will see the referees find the study describing a structure and functional data on the Spt6-Iws1 complex to be interesting and provide sufficient new insight to be further considered for publication in the EMBO Journal. Nevertheless they also raise a number of important issues regarding the text and figures that need to be addressed before the manuscript can be further considered for publication. Upon reflection these concerns are fair and central to the conclusions of the paper. Should be able to address these issues we would be happy to consider a revised version of the manuscript.

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

REFEREE COMMENTS

Referee #1 (Remarks to the Author):

Diebold et al. report the structure of the transcription elongation factor Iws1 bound to a short peptide of the elongation factor Spt6. The technical part is fine. Supporting biochemical data contribute to our understanding of the complex protein-protein interaction network that underlies gene transcription through chromatin. The manuscript will be suited for the EMBO Journal but requires major revision of the text and figures, as outlined below, before it can be considered for acceptance. The language must be corrected throughout. At least one of the authors is a native speaker and could do this easily.

1. Title: "reveal" must be "reveals". The complex is best described as a Iws1/Spt6N complex (not Spt6/Iws1) since it contains mainly Iws1 and only a very small N-terminal peptide of Spt6. This must be changed throughout. The word "specific" is meaningless in the context of the title and should be replaced by a term that describes the function of the domain.

2. Abstract: "Our four" must be deleted. "Iws1 and the Spt6/Iws1" must be "Iws1 and the Iws1/Spt6N". For the two domains some information must be included on their structure. Also, the two domains should be given names in the abstract and these must be used throughout. They are not always referred to with the same name and this is very confusing. In fact, I am not sure these are two independent domains, and, if indeed the two domains show extensive interactions, would prefer if the authors use the term "subdomains". Lines 8-10: "this domain" indicates incorrectly the Iws1 domain. Must be changed.

3. Page 5, line 8: "extends to the structural level": This must be deleted or rewritten since structure is generally more (not less) conserved than sequence.

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7. Page 13: How can it be that the two TFIIS domain I structures do not superimpose? Are they conserved on the level of sequence? Can the yeast sequence be threaded through the mouse structure to obtain fine packing of hydrophobic residues in the core? What is the quality of the yeast TFIIS domain I data? The authors must investigate this further and then say clearly that the yeast TFIIS domain I structure is incorrect if that turns out to be so. This is a key point since otherwise one of the key conclusions of the paper can not be drawn.

8. Page 15, end of 2nd paragraph. The authors should read a recently published paper on the genome-wide distribution of elongation factors including Iws1 and Spt6 (Mayer et al., NSMB Sept. 5, 2010 online) and try to interpret elongation factor occupancy profiles on genes based on the detected protein interactions. From this, a possible explanation could be added here. This reference also shows that Spt6 can be recruited to elongating RNAPII even when the SH2 domain is lacking. The authors should thus revise what they say on Page 3, Lines 21-23.
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11. Page 16, first sentence of 3rd paragraph. The structural similarity can not demonstrate that the one TFIIS structure is incorrect. Rather the authors should do what is suggested in comment 7 and then provide a definite statement.

12. Page 17, last paragraph: what are the "putative determinants of phosphoprotein recognition"? I think this conclusion is not justified based on the provided data. Text should be toned down.

13. The authors should mention the model organism(s) they used earlier, ideally in the abstract.

14. Page 6, line 21: (residues 314-144) must be changed to (residues 144-314). Check carefully for similar mistakes throughout.

15. Page 6, line 22 (Heading): Iws1 conserved region is formed by HEAT and ARM repeats. Change to "The conserved region of Iws1 is formed by HEAT and ARM repeats"

16. Pages 13 and 14, Paragraph 'The IR1-binding domain of Iws1 is structurally conserved in TFIIS': maybe this is out of scope for this paper, but it would be nice to show/investigate the in vivo effects of the mutations affecting the TFIIS/Med13 and TFIIS/Spt8 interactions, respectively

17. Fig. 1C legend should include: Iws1 degradation products are marked with '*'.

18. Structural figures should be improved by adding labels, for example for the two subdomains and the bronine ions. In general, many things that are not referred to in the text or are not central to the paper are labeled whereas other things that are important for understanding the text are not labeled in the figures. Please go through all figures and make sure the labels match to the text.

19. Fig 3: (A), (B) could indicate hydrogen bonds; move (C) to Supplemental

20. The schematic view in Figure 1A must be improved. Where are the regions and domains that were newly defined by the paper? Why not introducing Spt6N and indicating it here? The published domain organization of Spt6 is indicated, but none of the domains is explained in the legend. This has to be completed.

21. I recommend to show a real multiple sequence alignment of Iws1 of various organisms in the supplementary part of this manuscript.

22. It seems to me as if different types of asterisks were used in Figure 2B. Please assure to be consistent here. This is also true for the font sizes.

23. Since the binding interface between Iws1 and Spt6 represents an integral part of this manuscript all interactions should be shown and described well. I recommend to include a schematic representation of the interacting residues with all hydrogen bonds and hydrophobic interactions indicated. See Verdecia et al NSB 2000 (Figure 2C in this paper). This representation could replace Figures 3C, 3D and 3E. These three figures could be transferred to the Supplementary. Alternatively or additionally, a table with protein-protein contacts can be provided.

24. The idea of providing a model is fine. But the indicated model in Figure 7 is a conglomerate of various factors and complexes that is in its present form confusing. For example, one could order all factors around a nucleosomal DNA template. There must be a clear color code and labels that are consistent with other figures. Additionally, the message of the model has to be explained in more detail in the corresponding figure legend.

25. Page 3, Line 23: "retention of bulk poly(A)+ mRNAs" must be replaced by "retention of bulk poly(A)+ mRNAs in mammalian cells"

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29. Page 18, Line 21: For large-scale purification (...) It is again not clear if the protein purification refers to the crystallization trials.

30. Page 27, Line 12: The version of PYMOL used has to be included here.

Referee #2 (Remarks to the Author):

Spt6 and Iws1 are conserved and essential transcription regulators of eukaryotes. Spt6 is thought to be a histone chaperone and also appears to play roles in regulation of transcription elongation and RNA processing that may be distinct from its histone chaperone activity. Iws1 has been implicated in stimulation of transcription initiation in a step that occurs after recruitment of the TATA binding protein to promoters. In addition, proteomic studies have shown that Spt6 and Iws1 are binding partners.

This paper reports structural and functional characterization of the Spt6-Iws1 complex from E. cuniculi. These proteins show sufficient homology to the Spt6 and Iws1 proteins of the major model organisms to be informative and interesting to the transcription field at large. In addition, using insights derived from their Spt6-Iws1 structural work, the authors are able to describe the likely mode of interaction for several other important transcriptional regulators.

Little is known about the structure of Spt6. A partial structure of a bacterial protein that shares sequence similarity with Spt6 has been reported, but no eukaryotic Spt6 family member has yet been crystallized. In addition, little structure/function information has been reported for Spt6. Thus, with respect to Spt6, several novel insights are presented in this work:

-several distinct crystal structures are presented for the Iws1-Spt6 complex

-the Iws1-Spt6 interaction surface observed in these crystals is validated by in vitro binding data and by genetic data in yeast

-the crystal structure and mutagenesis data are used to considerably refine prior knowledge of the sites of interaction between Spt6 and Iws1

-the above data reveal a previously unappreciated conserved domain in the N-terminus of Spt6

Similar to Spt6, little has been known about the structure and function of Iws1. The interaction domain of human Iws1 with human Spt6 had been very crudely mapped to the C-terminal half of the protein, and a single mutation, K192N had been characterized in detail. In just the last two weeks, a paper has appeared online at the Journal of Molecular Biology, from the Stargel group, which describes the crystal structure of the isolated Iws1 protein.

The structure of Iws1 presented in this JMB paper spans approximately the same residues as in the structures presented in the manuscript under review. At least superficially, the structures seem to nearly identical. The authors of that paper note the structural similarity of TFIIS and Iws1 however, in the manuscript under review, this similarity is investigated in much greater detail and with the additional insights provided by the co-crystalization with Spt6. Thus, while the JMB paper is certainly a valuable addition to the literature, the manuscript under review has much more to offer.

Important new insights into Iws1 and Iws1-Spt6 interactions provided in the manuscript under review include:

- - elaboration and confirmation of structural details of the molecular interaction between Spt6 and Iws1
-
- the suggestion the cavity surrounding Iws1 residue K192 likely serves to bind a negatively

changed residue-potentially a phosphate. In the absence of a ligand for this cavity, this is a very interesting idea, but of uncertain significance. Have the authors constructed K192A, K192D and K192R iws1 alleles and analyzed them in yeast?
-
- - the recognition that the residues in Iws1that are conserved in TFIIS overlap the Spt6 binding site in Iws1
- - demonstration that TFIIS uses this domain to interact with Med13 and Spt8

In the text, the authors address a confusing conflict between the structures previously solved for the N-terminal domains of TFIIS from mice and yeast, by suggesting that the published yeast structure may be wrong. This conclusion certainly makes sense in terms of the data presented here. However, is the prior TFIIS data of sufficient quality to allow the authors to determine if the key conserved side chains implicated in Med13 and Spt8 binding would not be available if arranged in the published TFIIS sequence?

Overall, the paper is fairly complete, although the conclusions regarding the role of the K192 binding pocket will remain tentative until a ligand can be found for this residue.

Overall, the manuscript represents a clear step forward for the a broad swath of the transcription field, even in the light of the recent Iws1 structure in EMBO.

1st Revision - authors' response 14 October 2010

Referee #1 (Remarks to the Author):

Diebold et al. report the structure of the transcription elongation factor Iws1 bound to a short peptide of the elongation factor Spt6. The technical part is fine. Supporting biochemical data contribute to our understanding of the complex protein-protein interaction network that underlies gene transcription through chromatin. The manuscript will be suited for the EMBO Journal but requires major revision of the text and figures, as outlined below, before it can be considered for acceptance. The language must be corrected throughout. At least one of the authors is a native speaker and could do this easily.

- The correction of the language has been done.

1. Title: "reveal" must be "reveals". The complex is best described as a Iws1/Spt6N complex (not Spt6/Iws1) since it contains mainly Iws1 and only a very small N-terminal peptide of Spt6. This must be changed throughout. The word "specific" is meaningless in the context of the title and should be replaced by a term that describes the function of the domain.

- The title has been changed accordingly. Note however that the use of the word Spt6N within the title would be rather unusual and potentially confusing. Therefore we have kept Spt6 within the title. However, the suggestion of using Spt6N within the manuscript is a good idea and the name has been changed throughout the manuscript, the figures and the supplementary data, when applicable. Note also that, due to the changes required, the title slightly exceeds 100 characters.

2. Abstract: "Our four" must be deleted. "Iws1 and the Spt6/Iws1" must be "Iws1 and the Iws1/Spt6N". For the two domains some information must be included on their structure. Also, the two domains should be given names in the abstract and these must be used throughout. They are not always referred to with the same name and this is very confusing. In fact, I am not sure these are two independent domains, and, if indeed the two domains show extensive interactions, would prefer if the authors use the term "subdomains". Lines 8-10: "this domain" indicates incorrectly the Iws1 domain. Must be changed.

- The changes requested in the abstract have been made. For the two Iws1 domains, these have been renamed HEAT-subdomain and ARM-subdomain as suggested by the referee. These names are now being used throughout the manuscript, the figures and the supplementary data, clarifying indeed the manuscript.

3. Page 5, line 8: "extends to the structural level": This must be deleted or rewritten since structure is generally more (not less) conserved than sequence.

- This part of the sentence has been removed since it is not essential.

4. Page 5, line 11-12: This sentence cannot be understood, delete or rewrite.

- This sentence has been rewritten to clarify the message.

5. Page 9, line 1. The authors claim a bromine ion can mimic a phosphate ion. They must say why. Either provide a reference or argue based on coordination etc. The claim that the one Iws1 domain is a phospho-binding domain is not further supported by direct investigation.

- The paragraph describing the mimicking of a phosphate ion has been fully modified to include additional information (coordination, positioning of a coordinating water molecule that could be replaced by the oxygen of a phosphorylated residue) that argue in favour of the hypothesis of a phosphoprotein binding site

6. Page 10. The text must be shortened, unnecessary details should be skipped.

- The text of page 10 corresponds to the description of the interaction between Iws1 and Spt6N at the molecular level. We feel that this description is already at the minimum and we believe that shortening of this text will weaken the manuscript. Notably, the interactions described here are the basis for all the mutational work made afterwards. Since our manuscript is below the limit of the 55,000 characters, we do not see any reason to shorten this part of the manuscript.

7. Page 13: How can it be that the two TFIIS domain I structures do not superimpose? Are they conserved on the level of sequence? Can the yeast sequence be threaded through the mouse structure to obtain fine packing of hydrophobic residues in the core? What is the quality of the yeast TFIIS domain I data? The authors must investigate this further and then say clearly that the yeast TFIIS domain I structure is incorrect if that turns out to be so. This is a key point since otherwise one of the key conclusions of the paper can not be drawn.

- We cannot judge the quality of the NMR structure since the NMR restraints have not been deposited within the PDB. However, careful inspection of the structure reveals several charged residues with side chain oxygen or nitrogen atoms that are in hydrogen bonding distance with carbon atoms of hydrophobic residues, clearly indicating errors in the NMR model established. Therefore, as suggested by the referee, we have established a model of the yeast TFIIS Domain I based on the mouse structure using conventional modeling programs. Inspection of the model shows that the yeast TFIIS Domain I can perfectly adopt the same fold as the mouse TFIIS Domain I without any steric clashes or any unconventional structural feature, arguing once again against the initial structure. This model can also be perfectly superposed on our Iws1 structure. Taken together, although the ultimate proof of an incorrect structure would require ab initio structure determination of the yeast TFIIS Domain I, this analysis, together with the fact that we observe a mode of binding of Spt8 and Med13 to TFIIS which is similar to the binding of Spt6N to Iws1, very strongly argues for the incorrectness of the yeast structure. All this has now been clearly

stated within the manuscript, including the fact that the model can be obtained from us upon request.

8. Page 15, end of 2nd paragraph. The authors should read a recently published paper on the genome-wide distribution of elongation factors including Iws1 and Spt6 (Mayer et al., NSMB Sept. 5, 2010 online) and try to interpret elongation factor occupancy profiles on genes based on the detected protein interactions. From this, a possible explanation could be added here. This reference also shows that Spt6 can be recruited to elongating RNAPII even when the SH2 domain is lacking. The authors should thus revise what they say on Page 3, Lines 21-23.

- We are perfectly aware of this paper since we also solved the structure of the C-terminal domain of Spt6 and showed that it contains in fact a tandem SH2 domains (Diebold et al., JBC, in press). We understand from the comment of the referee that in vivo masking, as suggested in the Mayer paper, could explain our lack of characterization of a strong complex between Iws1 and the phosphorylated RNAPII CTD. In fact, our experiments have been done in vitro and cannot be compared with the results of the Mayer paper. The manuscript has been modified accordingly.
- Concerning the recruitment of Spt6 through its tandem SH2 domain by the phosphorylated RNAPII CTD, the Mayer paper suggests indeed that it may not be the only way to recruit Spt6 since this latter protein is still recruited to the body of genes in absence of its tandem SH2. However, in the Mayer paper the level of the Spt6DC mutant recruited is much less than in the case of the WT Spt6. We have independently made the same mutant for our own studies and showed that this mutant creates yeast strains that are extremely sick (Diebold et al., JBC, in press). We think that it cannot be excluded that this mutant is recruited by another factor that interacts directly with the polymerase. Although this recruitment may not be essential or may even not be biologically relevant in vivo, it could prevent the yeast cell from inviability in the presence of the mutant (Spt6 is an essential protein). We feel that as long as this discrepancy has not been further studied experimentally to discard any possible artefact, the introduction should not be revised. However, a sentence mentioning the Mayer paper has been added immediately afterwards to provide the reader with up-to-date information.

9. Page 15, line 1: I do not think that there is a wealth of data on Iws1. Should be rephrased.

- This has been done.

10. Page 16, last line of 2nd paragraph. Is there an interaction or not? Must be rephrased.

- This has been done.

11. Page 16, first sentence of 3rd paragraph. The structural similarity can not demonstrate that the one TFIIS structure is incorrect. Rather the authors should do what is suggested in comment 7 and then provide a definite statement.

- Due to the changes made to the manuscript in response to comment 7, we have decided to remove the second part of the sentence mentioned here since it is not important anymore.

12. Page 17, last paragraph: what are the "putative determinants of phosphoprotein recognition"? I think this conclusion is not justified based on the provided data. Text should be toned down.

- This has been done by stating that Iws1 HEAT-subdomain may recognize a phosphoprotein.

13. The authors should mention the model organism(s) they used earlier, ideally in the abstract.

- The abstract has been changed to include this information.

14. Page 6, line 21: (residues 314-144) must be changed to (residues 144-314). Check carefully for similar mistakes throughout.

- This has been done.

15. Page 6, line 22 (Heading): Iws1 conserved region is formed by HEAT and ARM repeats. Change to "The conserved region of Iws1 is formed by HEAT and ARM repeats"

- This has been done.

16. Pages 13 and 14, Paragraph 'The IR1-binding domain of Iws1 is structurally conserved in TFIIS': maybe this is out of scope for this paper, but it would be nice to show/investigate the in vivo effects of the mutations affecting the TFIIS/Med13 and TFIIS/Spt8 interactions, respectively

- We do agree with the referee but this is indeed out of scope for this paper.

17. Fig. 1C legend should include: Iws1 degradation products are marked with ''.*

- This has been added.

18. Structural figures should be improved by adding labels, for example for the two subdomains and the bronine ions. In general, many things that are not referred to in the text or are not central to the paper are labeled whereas other things that are important for understanding the text are not labeled in the figures. Please go through all figures and make sure the labels match to the text.

- Labels for the subdomains and the bromide ions have been added to the figures when required and figure legends have been modified accordingly. With a few exceptions that have been corrected, inspection of the text showed good agreement with the figure labels. However, it is true that sometimes more side chains (and labels) have been displayed in the figures as long as the legibility of these figures was not impacted. We have done so to provide the reader with more structural information without increasing the length of the text (in agreement with comment 6 of the referee).

19. Fig 3: (A), (B) could indicate hydrogen bonds; move (C) to Supplemental

- Adding hydrogen bonds worsen the legibility of the (A) and (B) panels. Therefore we have preferred to keep the figures 3A and 3B as they are.
- We believe that moving panel (C) to the supplemental data is not appropriate. Indeed, figure 3C contributes to the hypothesis of a phosphorylation recognition motif by the HEAT-subdomain, notably due to the large density observed for the bromide ion. Comment 5 of referee clearly highlights this requirement.

20. The schematic view in Figure 1A must be improved. Where are the regions and domains that were newly defined by the paper? Why not introducing Spt6N and indicating it here? The published domain organization of Spt6 is indicated, but none of the domains is explained in the legend. This has to be completed.

- This has been done. Both figure 1A and its legend have been fully modified taking into account the requests of the referee and the recent published data.

21. I recommend to show a real multiple sequence alignment of Iws1 of various organisms in the supplementary part of this manuscript.

- We do not understand the rational for this recommendation which is not provided by the referee. The alignment provided in supplementary figure 1B already gives a clear view of the conservation between human, yeast and *E. cuniculi* Iws1 proteins.

22. It seems to me as if different types of asterisks were used in Figure 2B. Please assure to be consistent here. This is also true for the font sizes.

- The font for the asterisks was indeed wrong. This has been changed.

23. Since the binding interface between Iws1 and Spt6 represents an integral part of this manuscript all interactions should be shown and described well. I recommend to include a schematic representation of the interacting residues with all hydrogen bonds and hydrophobic interactions indicated. See Verdecia et al NSB 2000 (Figure 2C in this paper). This representation could replace Figures 3C, 3D and 3E. These three figures could be transferred to the Supplementary. Alternatively or additionally, a table with protein-protein contacts can be provided.

- We are surprised of this request which contradicts comment 6 of the referee, asking to shorten the text on page 10 that describes the interactions between Spt6 and Iws1. Although there are two Verdecia papers in NSMB in 2000, in our eyes the figure 2C that we assume to be the one suggested by the referee does not provide more information than the current figures of our manuscript. Tables with protein-protein interactions are generally not anymore provided within manuscripts since the structures are released immediately upon manuscript acceptance. We are strongly convinced that the text on page 10 and the associated figure 4 (from the names of the panels we assumed that the referee meant Figure 4 rather than Figure 3) clearly convey the information on the complex formation.

24. The idea of providing a model is fine. But the indicated model in Figure 7 is a conglomerate of various factors and complexes that is in its present form confusing. For example, one could order all factors around a nucleosomal DNA template. There must be a clear color code and labels that are consistent with other figures. Additionally, the message of the model has to be explained in more detail in the corresponding figure legend.

- We have been making different versions of figure 7 before choosing the actual figure submitted. Specifically, we have indeed tried to organize the different molecules around nucleosomes but quickly realized that we were losing the focus of the manuscript on the Iws1/Spt6 complex. That explains our current choice. Note that we have also solved the structure of the full C-terminal domain of Spt6 (Diebold et al., JBC, in press) and we have updated the figure accordingly. As suggested by the referee, we have also changed the colours associated with the Iws1/Spt6N complex to be in agreement with the other figures. Finally, we agree with the referee that the figure brings together quite a few different structures. This reflects the complexity of the system studied; and figure 7 has been made exactly for this purpose of bringing all this information together in order to provide the reader with an up-to-date view of the Iws1/Spt6 complex. As suggested by the referee, the figure legend has been fully rewritten to clarify the different information provided by the figure.

25. Page 3, Line 23: "retention of bulk poly(A)+ mRNAs" must be replaced by "retention of bulk poly(A)+ mRNAs in mammalian cells"

- This has been made.

26. Page 13, Lines 10-12: Here it is stated that no satisfying fit was found with the yeast protein. The PDB code of the yeast TFIIS structure used should be included here.

- This has been done.

27. Page 18, Line 16: on the affinity resin (...) Which resin was used?

- This has been corrected.

28. Page 18, Line 19: For small-scale analysis (...) It is not clear if the interaction assay is meant?

- This has been clarified.

29. Page 18, Line 21: For large-scale purification (...) It is again not clear if the protein purification refers to the crystallization trials.

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Little is known about the structure of Spt6. A partial structure of a bacterial protein that shares sequence similarity with Spt6 has been reported, but no eukaryotic Spt6 family member has yet been crystallized. In addition, little structure/function information has been reported for Spt6. Thus, with respect to Spt6, several novel insights are presented in this work:

-several distinct crystal structures are presented for the Iws1-Spt6 complex

-the Iws1-Spt6 interaction surface observed in these crystals is validated by in vitro binding data and by genetic data in yeast

-the crystal structure and mutagenesis data are used to considerably refine prior knowledge of the sites of interaction between Spt6 and Iws1

-the above data reveal a previously unappreciated conserved domain in the N-terminus of Spt6

Similar to Spt6, little has been known about the structure and function of Iws1. The interaction domain of human Iws1 with human Spt6 had been very crudely mapped to the C-terminal half of the protein, and a single mutation, K192N had been characterized in detail. In just the last two weeks, a paper has appeared online at the Journal of Molecular Biology, from the Stargel group, which describes the crystal structure of the isolated Iws1 protein.

The structure of Iws1 presented in this JMB paper spans approximately the same residues as in the structures presented in the manuscript under review. At least superficially, the structures seem to nearly identical. The authors of that paper note the structural similarity of TFIIS and Iws1 however, in the manuscript under review, this similarity is investigated in much greater detail and with the additional insights provided by the co-crystalization with Spt6. Thus, while the JMB paper is certainly a valuable addition to the literature, the manuscript under review has much more to offer.

- We became aware of the JMB paper a couple of days before receiving the referee reports. This paper confirms by in vivo studies the importance of the Iws1-specific HEAT-subdomain that we have highlighted in our manuscript. The authors also raise several questions based on their structure. Our data and our manuscript provide answers to most of these questions. References to the JMB paper have been added within our manuscript.

Important new insights into Iws1 and Iws1-Spt6 interactions provided in the manuscript under review include:

- elaboration and confirmation of structural details of the molecular interaction between Spt6 and Iws1

- the suggestion the cavity surrounding Iws1 residue K192 likely serves to bind a negatively changed residue-potentially a phosphate. In the absence of a ligand for this cavity, this is a very interesting idea, but of uncertain significance. Have the authors constructed K192A, K192D and K192R iws1 alleles and analyzed them in yeast?

- We have not made these mutants since the K192N mutant had already been thoroughly characterized. However, it will be important to do this kind of experiment once the CTD binding to the HEAT-subdomain of Iws1 has been clearly identified.

- the recognition that the residues in Iws1that are conserved in TFIIS overlap the Spt6 binding site in Iws1

- demonstration that TFIIS uses this domain to interact with Med13 and Spt8

In the text, the authors address a confusing conflict between the structures previously solved for the N-terminal domains of TFIIS from mice and yeast, by suggesting that the published yeast structure may be wrong. This conclusion certainly makes sense in terms of the data presented here. However, is the prior TFIIS data of sufficient quality to allow the authors to determine if the key conserved side chains implicated in Med13 and Spt8 binding would not be available if arranged in the published TFIIS sequence?

- Please refer to the changes made when addressing comment 7 of referee 1. These changes and our answer to the comment fully address the issues raised here by referee 2.

Overall, the paper is fairly complete, although the conclusions regarding the role of the K192 binding pocket will remain tentative until a ligand can be found for this residue.

Overall, the manuscript represents a clear step forward for the a broad swath of the transcription field, even in the light of the recent Iws1 structure in JMB.

2nd Editorial Decision 15 October 2010

Thank you for submitting a revised version of your manuscript to the EMBO Journal. I have had an opportunity to look through your response to the referees concerns and find that you have satisfactorily addressed the issues raised. I am happy to accept your manuscript for publication in The EMBO Journal, you will receive the official acceptance letter early next week.

Sincerely yours

Editor

