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Molecular insights into the recruitment of TFIIH to sites of DNA damage

Valentyn Oksenych, Bruno Bernardes de Jesus, Alexander Zhovmer, Jean-Marc Egly

Corresponding author: Frederic Coin, IGBMC

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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 31 March 2009

Thank you for submitting your manuscript for consideration by the EMBO Journal. I have now had a chance to read it carefully and to discuss it with my colleagues. In addition, I decided to consult one of our external advisors. Following these discussions, I am sorry to inform you that our decision is a negative one, and we can not offer to publish your manuscript.

We appreciate that you have further investigated the molecular function of the TFIIH helicase XP-B. Your have previously shown that the helicase activity of XP-B is dispensable for its function in nucleotide excision repair, whereas the ATPase activity is essential. Here, you confirm and extend these results, showing that ATPase inactive XP-B is not recruited to sites of DNA damage. You then investigate various domains and motifs of XP-B, showing that the Thumb and RED motifs are required for XP-B activity, whereas the DRD is not - contrary to previous results implicating the DRD in damage recognition by XP-B. From these results, you then propose a model whereby XP-B acts as an ATPase dependent anchor for the TFIIH complex, interacting with chromatin via its Thumb and RED motifs, and allowing access for the XP-D helicase. While we do appreciate the importance of understanding the function of XP-B, we find that your current study does not provide sufficient direct evidence for the model you propose: while your assays clearly demonstrate that the Thumb and RED motifs are important, they do not further elucidate the mechanistic details of XP-B action, either in terms of their effects on ATP hydrolysis or on mediating chromatin association. These concerns as to a lack of mechanistic depth were shared by our external advisor, whom I had contacted in order to gain an expert opinion on your manuscript. For these reasons, I am afraid we feel that your manuscript does not make the kind of conceptual advance required for us to consider it

further for publication in the EMBO Journal.

Please note that we publish only a small percentage of the many manuscripts that we receive at the EMBO Journal, and that the editors have been instructed to only subject those manuscripts to external review which are likely to receive enthusiastic responses from our reviewers and readers. I am sorry to have to disappoint you on this occasion, but I hope that this negative decision will not prevent you from considering the EMBO Journal for publication of future studies.

Rebuttal Letter 15 April 2009

Bonjour,

I am writing concerning the last paper we have submitted (Manuscript EMBOJ-2009-70932). "You do not offer to publish your manuscript" as you said but it could have been worthwhile to send it for reviewing. I belong to your board and I know the criteria of EMBO journal and I think that our paper could have been taken into consideration and send to review. Indeed and contrary to what was assessed by one of your external advisor, this paper clearly shows that the functions of XPB and XPD ATP dependent helicases of TFIIH can be discriminated during DNA repair: Upon UV irradiation and once XPC has recognized the damaged site, TFIIH arrives:

1) the ATPase activity developped by XPB allows the recruitment of TFIIH at the damaged site. In such case the XPB helicase is not required.
2) the ATP hydrolysis of XPD allows the unwinding and further the opening of the DNA by TFIIH around the damaged site, and then the recruitment of the additional NER factors.

More interestingly, is the observation that XPB possess what can be named "ATPase motifs", that help (or regulate) the ATPase activity and therefore allow TFIIH to develop an optimal ATPase activity. We show that mutations in these two motifs prevent the binding of TFIIH to the damaged DNA not only in vitro but also in vivo. Additional assays performed last week show that these mutant did not allow optimal stimulation of the ATPase activity in the presence of DNA, thereby explaining to the molecular level the impact of these motifs.

I guess that this paper will oblige us to change our idea (as well as all the cartoons designed in the literature), that the XPB and XPD helicase had the same function in NER : unwind the DNA on 5' and 3' on each side of the damage. I guess that this is an important contribution which follows previous works (Riedl et al. EMBO, 2003; Mocquet et al., EMBO, 2007), describes and precizes the NER mecanism. I am ready to discuss it at the phone at your earlier convenience.

Salutations

PS I also send this e-mail to your colleague who was familliar with our work

Response to Rebuttal 16 April 2009

Many thanks for your correspondence regarding your recent submission (EMBOJ- 2009-70932). I have now had the chance to consider your rebuttal, and have discussed your case further with my colleagues, and with our executive editor Pernille Rorth. Following these discussions, we are willing to send your manuscript out for formal peer review, should you still wish us to do so. I should add that our decision to reconsider your manuscript at this stage does not, of course, provide any guarantee as to the outcome of the review process.

You mentioned additional experiments in your rebuttal letter and it is unclear to me whether you want to include these in your current submission or not. Please can you let me know either way so that we can proceed appropriately - it is of course no problem to submit a revised version if you feel this would improve the manuscript.

Response by Author 17 April 2009

We appreciate very much that you reconsidered your decision and gave this paper a chance to be reviewed. As we have improved the paper by adding some new experiments taking into account your comments, we think it will be better to submit a new version of our manuscript. That this submission is a new one or a revised version of EMBO2009-70932 depends on you, just tell us how to proceed online and we will act accordingly.

2nd Editorial Decision 02 June 2009

Thank you for resubmitting your manuscript for consideration by the EMBO Journal. As agreed, I sent it out for in-depth peer review, and have now heard back from the three referees, whose comments are enclosed. As you will see, all three express interest in your results, and are broadly supportive of publication - contingent upon a number of concerns being resolved. In particular, I would like to draw your attention to the comments of referee 1 (points 2 and 3) regarding some of the cellular assays, for which better quantification is required.

Given the referees' positive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing all the comments of all three reviewers. I should add that it is EMBO Journal policy to allow only a single round of revision. Acceptance of your manuscript will thus 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.

REFEREE REPORTS:

Referee #1 (Remarks to the Author):

Our present understanding of human nucleotide excision repair (NER) is that XPC-HR23B is the initial damage recognition factor that recruits TFIIH to sites of DNA damage. TFIIH contains two helicases, XPB and XPD. While ATPase activity of both XPB and XPD is needed for NER, only the helicase activity of XPD, but not XPB is required for NER. Here Oksenych and colleagues show that two functional motifs of XPB, RED and ThM - originally discovered in structural studies of an archeal XPB homolog, as well as its ATPase activity are required to anchor XPB in DNA in vitro and in vivo in the context of NER and transcription. By contrast, the ATPase activity of XPD is not needed to recruit TFIIH to sites of UV damage. The implications of this work are that recruitment of TFIIH to sites of NER requires functional XPB protein, while the ATPase/helicase activity of XPD is only needed at later step. These finding represent an important advance in our understanding of the damage recognition/verification steps. Prior the publication, the writing throughout the manuscript should be improved and several major points need to be addressed:

1) The discovery of the RED, THUMB and DRD domains stems from structural studies by John Tainer and colleagues of an archeal homolog of XPD (Fan et al, Mol Cell 2006). This paper forms the basis of the studies described here. Although the Fan paper is referenced, it needs to be discussed more prominently in i) the abstract, ii) the introduction and iii) in the legend to Figure 2 and iv) in the discussion.

2) The survival data in Figure 3C need to be described better: i) there is no explanation in the experimental section of how these experiments were done; ii) error bars are need to show that the difference in survival at the 9hr time point is statistically significant. iii) it is unclear whether the scale of the y axis (0.1-1%) is logarithmic or linear.

3) In Figure 5 it is shown that in cells expressing XPB-E473A and XPB- 516-526 TFIIH is not recruited to sites of UV damage. Inspection of the Figure 5e-h and q-t would suggest that XPB-E253A/R283A and XPB-T469A colocalizes with CPDs in some cells, but no in others. The authors conclude from this that the DNA recognition and helicase domains of XPB are not required for

recruitment of TFIIH to sites of CPD. This conclusion is not sustained by the data, unless appropriate statistics are provided for Figure 5, indicating in what percentage of the different cells CPDs colocalizes with TFIIH.

The following minor points should be addressed:

4) There is no mention in the text that a XPB-GFP construct was used - A reference should be included demonstrating that this fusion construct is active

5) The discussion on page 10, line 5 should reference the following paper as the first one to identify XPC as the first damage recognition factor: Sugasawa et al, Mol Cell, 2, 223 (1998), and Yokoi et al JBC, 275, 9870 (2000)in line 6 as the paper to show that TFIIH is recruited by interaction with XPC.

Referee #2 (Remarks to the Author):

TFIIH is a multi-functional ten-subunit protein complex that is essential for both transcription and nucleotide excision repair. It contains two helicases of opposite polarity and understanding the roles and coordination of these two proteins is critical for understanding the role of the entire complex in the cell. The importance of these helicases is underscored by three human diseases that result from defects in XPB and XPD. Since the phenotypes of these diseases include both premature cancer and premature aging, understanding the roles of XPB and XPD is vital for human health.

This manuscript seeks to understand the roles of XPB and XPD by first analyzing the localization of mutant XPB and XPD in CHO cells by confocal microscopy. Although an ATPase mutant of XPD blocks incision and cannot open DNA, it localizes to local UV damage. This is in contrast to an XPB ATPase mutant that does not localize to UV damage in the cell as well as blocking NER incision and defective in DNA opening. These initial results suggest that only XPB must be functional for damage localization.

The authors then explore the roles of three motifs identified by published structural analysis of an archaeal XPB homolog and proposed to be important in its function: 1) a putative damage recognition domain (DRD), 2) a loop containing R-E-D residues proposed to be important in DNA unwinding, and 3) a Thumb (ThM) domain proposed to aid in DNA binding. The authors use a variety of complementary methods to assess the role of these motifs for XPB function including transcription, incision, footprinting, reporter, and survival assays as well as localization by confocal microscopy. They report that both the RED and ThM motifs are important for XPB function, as is ATPase activity of XPB. They do not see an effect of mutating the DRD domain using any of the endpoints tested.

Overall, the manuscript is clear and well-written. The data is presently logically and concisely. The experiments are straightforward and the results are significant. It is an excellent example of what can be learned when cell biologists test hypotheses proposed by structural biologists. In this case, ATP hydrolysis was proposed to drive a large conformational change in XPB that would bring the RED motif and ThM thumb domain in close proximity to then grip and unwind the DNA. The experiments presented in this manuscript confirm that ATPase activity, the RED motif, and the ThM domain are indeed important for XPB function in the cell. In contrast, the DRD domain was found to not function as proposed based upon its structurally similarity to the MutS damage recognition domain, suggesting that how TFIIH recognizes damage is still an open question. Before publication, the authors should consider the following points.

Points.

1. Methods or references for methods are spread throughout the Results and Materials and Methods sections as well as in the figure legends. Although some experimental detail is provided in the figure legends, it would be helpful to provide brief sections in the Materials and Methods for each assay so that a consistent level of information is provided for each.

2. Figure 1A legend - "TIIH/XPB(K346R)" should read "TFIIH/XPB(K346R)"

3. Figure 2B legend - "The position of the new mutations is indicated" should read "The positions of the new mutations are indicated"

Referee #3 (Remarks to the Author):

The process by which DNA repair proteins find their damaged nucleotide substrates is a fundamental problem. In this study the authors have mutated amino acid residues in the two helicase domains of TFIIH a protein machine involved in transcription initiation and nucleotide excision repair (NER). They first show that mutating a critical lysine residue in the ATP binding site of either XPD or XPB abolishes NER and opening around a lesion. TFIIH containing XPD (K48R) is capable of supporting transcription, and while deficient in supporting incision, is shown to be recruited at UV-induced CPD. This is contrast to XPB(K346R) which is deficient all three activities. The authors then go on to examine four additional mutations in XPB: E253, R283A, E473A and delta516-526. The E473A mutation in a hairpin they call the RED domain, and the delta516-526 mutation (part of a putative Thumb domain) behave similarly to the K346R mutant, in that they do not support transcription, or incision, and are not recruited at UV-induced CPD. Finally they show that that these three mutants are defective in ATPase activity in the presence of DNA. The authors conclude that these residues and domains of XPB are important for helping to target TFIIH to promoters and sites of DNA damage through its ATPase activity. This is a potentially important contribution with wide reaching implications, once the authors have responded to the following concerns.

1. The quality of English usage is not up to the standards of the journal. For example on page 2 of the abstract the authors state, "Besides, we show.." ; on page 6, "... demonstrated lately..."; page 11 "... to the promoters and to the damages..."; on page 17 Figure legend 2, "localization" should simply be "location".
2. There is some redundancy in the experimental results. The experiments in Figure 1 are important, but the authors show similar experiments with the same mutant, K346R later in Figure 4. Also the authors show the same information as has been previously published for T469A in Figure 5.
3. Experiments shown in Figure 1 (Panel C) and Figure 5 show a three way color merge, which and it might be difficult to discern the white spots (true CPD and XPB subsition) versus blue spots. Very often in confocal experiments the percent overlap or merge of the two colors are given. This would be very helpful to the reader.
4. Please give the nature of the damaged substrate used in Figure1 Panel A in the legend; is it the same as Panel B?
5. Text of Figure 2, the authors are encouraged to give place putative before "damage recognition" as they do in the text. This proposed role has not been verified by experimental evidence.
6. Perhaps a summary model conveying the role of these domains in target location for either a promoter or site of damage would be helpful, as the text on page 11 is not sufficient to fully appreciate the proposed transition in the structure.
7. The level of incision in Figure 4 is a bit misleading as it would appear that in the left panel that the K346R mutant has activity producing a doublet at nt 34 and 33; yet in the right panel this activity is much less robust. Further more the WT protein also produces bands that roun at 31 and 30 and would seem to be the actually incision products. Thus the authors are encouraged to show quantitation of these bands and a control in which no TFIIH is added, much like what is shown for Panel C in Figure 4. It would appear the authors are discounting the bands at 33 and 34 - please clarify.
8. On page 6 the authors talk about several mutants, simply saying four and listing would be helpful.
9. Bottom of page 8 the authors do not show data, on the delta 516-536 mutant of XPB. The manuscript in its present form is not that long and the authors should show these data. Perhaps these data were not added, since the overall expression of this mutant seems lower in the complentation assay, and perhaps is somewhat unfolded and therefore less stable. The authors are encouraged to add these data and resolve this issue.
10. Another reason the two XPB mutants are not capable of tracking to the damaged site would be a loss of interaction with XPC/hRAD23B heterodimer, although this interaction is believe to occur through XPD. Can XPC/hRAD23B still interact with TFIIH containing these XPB mutants? This could be done either by pull down or indirectly by showing that repair is arrested once XPC/hRAD23B is recruited to the site of the damage, but TFIIH containing these mutations are not

2nd Revision - Authors' Response 24 June 2009

We thank the referees for their positive and constructive comments on our manuscript.

Referee 1
1. We now mention and discuss the paper of John in the abstract, introduction, Figure legend 2 and in the discussion.
2. We now include errors bars in the Figure 3C. The survival assay has been described better in the experimental section. It is now mentioned in the figure that the y axis is logarithmic.
3. We now include both in the Figures 1 and 5 statistical data for our confocal experiments. We determined the percentage of CPD spot that co-localize with TFIIH in cells that expressed the human XPB or XPD proteins. In some cells the CPD do not co-localize with the TFIIH because the hXPB is not expressed (no nuclear staining of XPB or XPD can be observed).
4. We included a reference to answer to that point. Please, note that in Figure 3C we show that XPB-GFP restores the survival of CHO27-1 and is therefore functional.
5. The referee is right and the references have been added to the text.

Referee 2
1. We re-organized the experimental section to include materials and methods that were missing
2 and 3. Text has been changed accordingly

Referee 3
1. The paper has been proofread.
2. We think it is important to show that the repair activity of RED and ThM mutants falls into the range of the K346R in the same figure, but we agree that there was some redundancy in Figure 4A and B. Accordingly, we have redone these figure panels in a unique panel 4A. We agree that the functionality of the T469A mutant in NER was shown in 2007, however we didn't show in the aforementioned paper that TFIIH(T469A) co-localizes with CPD.
3. We now include both in Figure 1 and 5 statistical data.
4. This information has been included in the experimental section.
5. This has been done.
6. Following the refereeís suggestion, a model is presented in Figure 7.
7. As stated in 2, the figure panels 2A and 2B have been redone and quantification included. We apologize for the quality of the NER assay we presented in the first version. The quality of the damaged DNA may explain the presence of this doublet at nt 34 and 33 in some of our assay but these are not NER specific products.
8. This has been done.
9. We didn't want to saturate the readers with redundant information in this figure. TFIIH/XPB (516-526) behaves exactly like TFIIH/XPB(E473A) in all the assays we tested including the ATPase +/-DNA and to include these data in Figure 6 would overwhelm the figure and dilute the main message. However, we now show these data in Supplementary Data 1 to satisfy the referee.
10. In Bernardes et al. 2008, we demonstrated that XPC interacts with the XPB and p62 subunits of TFIIH. We showed that the interaction between XPC and XPB was mediated by the N-terminal part of XPB (first 40 amino acids). This interacting domain does not encompass the residues of the RED or ThM motifs and interaction with XPC is not impaired by mutations in these motifs.

3rd Editorial Decision 17 July 2009

Many thanks for submitting the revised version of your manuscript EMBOJ-2009-70932R1. It has now been seen again by referee 1, who is satisfied with the your responses to the reviewers' comments, and now finds it acceptable for publication.

