THE
EMBO
JOURNAL

Manuscript EMBO-2016-94920

A new sub-pathway of long-patch base excision repair involving 5' gap formation

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Review timeline:

Submission date:	31 May 2016
Editorial Decision:	04 July 2016
Revision received:	01 December 2016
Editorial Decision:	02 January 2017
Revision received:	07 March 2017
Accepted:	09 March 2017

Editor: Hartmut Vodermaier

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 04 July 2016

Thank you for submitting your manuscript on potential 5' patch formation during base excision repair. I am sorry for the delay in getting back to you with a decision, but after receiving comments from three expert referees, we considered it important to enter additional consultations between the referees and editors in order to determine whether the study would be a compelling candidate for an EMBO Journal article. The main issue, as you will see from the three reports copied below, is whether the repair processes observed and characterized in the present manuscript really constitutes a bona fide form of BER, or even an essential step in canonical LP-BER. These concerns are most explicitly voiced in the report of referee 1, who further expanded on his/her doubts in the following cross-consultations, but are to some extent also shared by referees 2 and 3. The latter two reviewers additionally raise various technical issues, but also list a number of queries regarding the functional/physiological importance of the new PARP/RECQ1-involving mechanisms.

Given the potential importance of a previously overlooked new (sub-)pathway such as the one described here, we would be willing to give you an opportunity to address the referees' concerns in a revised version of the manuscript. It is clear that this will require a substantial amount of additional time and experimental effort, and given the currently unclear outcome of the required revision work I am in the present case also not able to make strong predictions on the eventual outcome of re-evaluation by the referees; however, in light of the possibly broad significance of your conclusions I feel it will be crucial to corroborate them with strong and decisive further evidence. In any case, should you be able to strengthen the support for the functional importance of 5' patch formation in

base excision repair and to offer decisive insights into its relative contribution as compared to other cellular mechanisms (by following the constructive suggestions of especially reviewers 2 and 3), we should be happy to consider this work further for publication in The EMBO Journal.

REFEREE REPORTS

Referee #1:

The authors of the manuscript use a method that they have developed for studying in-cell base excision repair (BER) and aim to investigate the long-patch base excision repair pathway. In brief, the method involves several major steps:
1. Construction of single-lesion M13mp18 phagemid DNA
2. Transfection of this DNA into mismatch repair-deficient mammalian cells for DNA repair
3. Recovery of repaired DNA followed by restriction and transfection into bacterial cells.
4. Counting transformants to estimate repair efficiency.

By introducing mismatches into the constructs at different distances from the target lesion, the authors monitor repair. My major concern is that the method itself is provoking formation of an artificial substrate which is not repaired by BER, but is instead repaired by other means. Introduction of one mismatched base pair will melt several base pairs on each side of the mismatch at 37oC (Yan Zeng and Giovanni Zocchi. Mismatches and Bubbles in DNA Biophys J. 2006, 90: 4522-4529). Consequently, as long as the DNA single strand break is formed during base excision repair of the target lesion in mammalian cells, DNA will melt and form a "BER unrepairable" structure. This may be repaired as is suggested by the authors, however, it is not really long-patch BER.

[additional comments during cross-consultation:]

As I mentioned in my review, the major problem is not repair of mismatches, but formation of an artificial substrate after the incision is made by BER enzymes. Mismatches only contribute to the melting of the substrate, generating a new structure which is then repaired differently. This is, therefore, not BER as is claimed by the authors. In-cell experiments that the authors use as evidence to claim that this is a new BER pathway that operates in living cells, have also been carried out using the mismatch-containing substrate (Figure 1). In vitro reconstitution by itself does not prove the existence of the new pathway (Figure 2). The rest of the experiments are based on the original finding presented in Figure 1, thus the interpretation of these data may be questioned as well.

Referee #2:

In this study, the authors provide evidence for a novel reaction in the repair of base adducts by BER. Specifically they show that under certain circumstances, a 8 mer oligonucleotide is removed after base removal and strand incision by the combined actions of PARP1, RECQ1, RPA and ERCC1/XPF incision 5' to the lesion. This is an interesting and novel reaction but the manuscript title "5' patch formation in base excision repair" is not very informative and should be changed to provide more insight into the findings in the paper. There are also other areas were the study should be strengthened;

1) As noted by the authors, short and long patch pathways of BER have been defined, mainly in cell extract and reconstitution assays. One of the discrepancies between this study and published work in the size of the repair patch. Previous work suggested the long patch involved patches ranging from 2-12 nucleotides. The data shown in Fig. 1B are consistent with the removal of the 8 mer 5' oligonucleotide but it is not clear what the length of the repair synthesis is 3' to the lesion. From Fig. 1B it appears to be at least 11 nucleotides. The extent of repair synthesis 3' to the lesion should be determined as this will determine the total size of the repair patch.

2) All siRNA experiemnts are only done with one siRNA and there are no experiments showing complementation of knockdown effects by expression of an siRNA-resistant cDNA. In addition, studies with knockout cell lines do not include controls in which the KO cell is complemented by expression of the appropriate cDNA

3) Fig. 2B. The predicted 2.2 kb band is not visible. The studies with the 3' probe need to be explained in the Table shown in Fig. 2A.

4) As noted above, there appear to be some discrepancies regarding the size of the repair synthesis patch generated during long patch. The authors provides compelling data in Fig. 3B regarding the activity of RECQ1, PARP1 and ERCC1-XPF. The analysis of repair patches generated as a result of the inclusion of Pol delta, RFC and PCNA in similar reaction in the presence or absence of RECQ1, PARP1 and ERCC1-XPF would provide insights into this issue and provide support for the authors' speculation.

5) The authors present intriguing data that RECQ1 inhibits PARP1 activation. This effect is seen at low but not high NAD concentrations. There is no discussion of how this effect relates to physiological levels of NAD.

6) The results of the pull down assays shown in Fig. 3E are not convincing and lack controls ie no DNA and non-biotinylated DNA. An EMSA may be more informative in terms of identifying DNA-protein complexes.

7) The authors provide evidence that RECQ1-dependent long patch BER is the major activity in cells. This consistent with other cell-based studies showing that long patch BER predominates over short patch BER. There is evidence for replication-associated BER that is assumed to occur via long patch. Is the RECQ1-dependent long patch BER pathway operational in non-diving or G1 cells?

Referee #3:

The experiments here explored the repair of an etheno-A adduct placed within a defined DNA substrate that, in some instances, harbored a site-specifically introduced mismatch. The authors' investigations uncovered a novel mechanism of repair, which engages proteins of BER (namely MPG and APE1), NER and SSBR. Interestingly, the new repair process was found to involve RECQ1, a 3' to 5' helicase described to mediate the formation of a 5' 9 nt gap in cooperation with PARP1, RPA and XPF/ERCC1 (but not POLB or XPA) during the repair response; replicative polymerase(s) and FEN1 function to complete the process. The selection of this particular repair event is shown to be directed by RECQ1's regulation of PARP1 ADP-ribosylation activity. Additional experiments indicate that such repair may also be carried out on common oxidative DNA lesions, namely 8-oxoG and 5-OHU. While it seems shocking that this pathway was missed previously, the studies, which involve both cell-based and biochemical approaches, are comprehensively executed, and the conclusions largely justified. My recommendations for improvement include the following:

Major issues

1. Despite the arguments presented, I'm still uncomfortable with referring loosely to this newly described process as BER (or LP-BER). Yes, it involves a DNA glycosylase and APE1, but otherwise deviates from what has been traditionally thought of as BER, both SP/SN-BER and LP-BER. As the authors point out, prior studies have reported a patch size of 6-7 nts for LP-BER (see Discussion), which is incompatible with their model. Thus, to distinguish the new process from the previously described BER sub-pathways, the authors are encouraged to consider an alternative nomenclature, such as perhaps "5' gap LP-BER".
2. It's hard (for me) to gauge how much of the total repair involves the new mechanism of 5' gap LP-BER versus the more traditional pathways. For example, can the authors estimate the percentage of etheno-A lesions that are processed via the new repair response (relative to classic BER mechanisms), and how is this percentage derived? Indeed, it's not totally clear what "percent repair" means, and as such, the authors are encouraged to spend a bit more time describing the details of the

assay.
3. I think it would be worthwhile for the authors to point out that, in addition to the location of the damage and relative position of the mismatch varying, the substrates in many instances possess a different sequence context as well, potentially influencing the outcome. There may also be sequence effects on pathway selection, possibly explaining the differences with prior studies. Perhaps some comment on this thought can be included.
4. In some of the experiments, it's unclear what plasmid was used, making it difficult to interpret the results. See for example Figure 1E-H. While some assumptions can be made, the precise substrate should be stated explicitly in the legend on each occurrence.
5. It would be interesting to directly examine the participation of other 3' to 5' helicases, to determine the specificity of RECQ1 for the observed 5' gap-BER event. For example, have the other human RECQ paralogs (WRN, BLM) been evaluated using a knockdown approach?
6. Similarly, it would be better to more directly assess the involvement of MUS81 by using a knockdown strategy. Alternatively, the discussion of the MUS81 expression should be treated more cautiously, as the results are by no means definitive regarding its participation as an alternative enzyme in the process.
7. The images of Figure 3E are not clear in several instances. The results would be strengthened by better images or by quantitation of the blots.
8. Given the degree of the effect of RECQ1 knockdown on 8oxoG repair and SSB accumulation (Fig 5), it seems surprising that knockdown has such a mild effect on cell survival. The authors should consider comparing the DNA damage levels and/or survival of OGG1-knockdown, RECQ1-knockdown and double-knockdown cells to get a better sense of the relationship between these two components.
9. What is known about the sensitivity of XPF or ERCC1-deficient cells to classic BER DNA-damaging agents, such as MMS or hydrogen peroxide? One would expect increased sensitivity as a function of its role in 5' gap LP-BER, and some discussion along these lines might be useful
10. The statement in the Discussion "which we have identified as RECQ1-dependent LP-BER" is inaccurate, as the authors (as far as I can tell) have not conducted studies using a uracil-containing substrate (OHU is not the same). Either the experiments need to be performed, or the sentence should be modified accordingly.

Minor issues
When printed, the text in many of the figures is very difficult to see, even with my 1.5X reading glasses.
Figure 1A. NA and ND need to be defined in the legend.

1st Revision - authors' response 01 December 2016

Responses to reviewers:

Reviewer #1:
We appreciate the reviewer's concern that the introduction of mismatches could lead to the formation of an artificial substrate that is not repaired by BER. However, we believe that we can mitigate his/her concern with two pieces of data from the original submission.

1. In Figure EV1, we exhaustively probe for the roles of various bona fide members of the BER pathway, including MPG (Zharkov & Grollman, 2005), APE1 (Wilson & Barsky, 2001), POLβ (Matsumoto & Kim, 1995), POLδ/ε (Klungland & Lindahl, 1997; Pascucci et al, 1999), and FEN1 (Klungland & Lindahl, 1997), in repair of εA on a plasmid containing a 5' mismatch. It is clear from the results of these experiments that MPG, APE1, POLδ/ε, and FEN1 are required for resolution of not only the lesion but also the neighboring mismatch. Since BER enzymes both before AP-site incision (MPG, APE1) and after AP-site incision (POLβ, POLδ/ε, FEN1) were required for resolution of εA and the neighboring mismatch, we conclude, therefore, that BER is the pathway responsible for the repair we are detecting in cells and that the original substrate (lesion + neighboring mismatch) and any intermediate substrate (single strand break + neighboring mismatch) are both BER repairable substrates.

2. In Appendix Figure S5A, we present the results of the plasmid-based assay for repair of εA without the neighboring mismatch. Of course, using our strategy, without the mismatch we cannot

then probe for patch formation on the 5' or 3' sides, but importantly, knockdown of RECQ1 in the experiment abrogated repair to the same extent as experiments performed with the mismatch (15-20% total repair in absence of RECQ1 in 24 h; see Figure 1E). Therefore, RECQ1 is required for repair of εA whether or not a neighboring mismatch is present and should be considered then to be operating as part of the BER pathway.

Reviewer #2:
* Reviewer #2 suggested a title change along with the comments below to strengthen the study."5' gap long patch base excision repair"

1. "As noted by the authors, short and long patch pathways of BER have been defined, mainly in cell extract and reconstitution assays. One of the discrepancies between this study and published work in the size of the repair patch. Previous work suggested the long patch involved patches ranging from 2-12 nucleotides. The data shown in Fig. 1B are consistent with the removal of the 8 mer 5' oligonucleotide but it is not clear what the length of the repair synthesis is 3' to the lesion. From Fig. 1B it appears to be at least 11 nucleotides. The extent of repair synthesis 3' to the lesion should be determined as this will determine the total size of the repair patch."

4. "As noted above, there appear to be some discrepancies regarding the size of the repair synthesis patch generated during long patch. The authors provides compelling data in Fig. 3B regarding the activity of RECQ1, PARP1 and ERCC1-XPF. The analysis of repair patches generated as a result of the inclusion of Pol delta, RFC and PCNA in similar reaction in the presence or absence of RECQ1, PARP1 and ERCC1-XPF would provide insights into this issue and provide support for the authors' speculation. "

Response to comments 1 and 4: We thank the reviewer for these suggestions to strengthen the study. To provide more information regarding the total size of the repair synthesis patch, we have added the full analysis of the 3' repair patch in the revised manuscript by finely mapping the 3' patch at positions +12 and +13 (Figure 1B) using our in-cell repair assay. Interestingly, we found patch formation up to 12 nts on the 3' side, which is consistent with other LP-BER studies (Sattler et al, 2003). This new data has led us to conclude that the total patch size in 5' gap-mediated LP-BER is 21 nts (9 nts on the 5' side (Figure 3B) and 12 nts on the 3' side (Figure 1B)).

We believe that our fine mapping of the repair patch by the in-cell repair assay addresses the concern noted in comment #4 related to discrepancies in repair patch size. Regarding the reviewer's question about the roles of individual gap formation proteins in repair patch synthesis/size, we have shown in Figures 1E and EV2A that knockdown of RECQ1, a major player in 5' gap-mediated LP-BER, abolished 5' patch formation as well as 3' patch formation, confirming that RECQ1 is required for total repair patch synthesis in the cells. We would like to note that the scope of our study was to elucidate the 5' gap formation step of this new LP-BER sub-pathway, so we did not investigate the activities of the repair synthesis machinery. A full investigation into the relationship between gap formation enzymes and the gap synthesis enzymes presents new and interesting challenges because of the novelty of the 5' gap formation step, and we believe such an investigation deserves of its own detailed study in the future, which we will be enthusiastically pursuing.

2. "All siRNA experiments are only done with one siRNA and there are no experiments showing complementation of knockdown effects by expression of an siRNA-resistant cDNA. In addition, studies with knockout cell lines do not include controls in which the KO cell is complemented by expression of the appropriate cDNA"

Response: We thank the reviewer for noting these missing controls which will strengthen our findings. We have revised the study by including at least 3 independent tests of the involvement of the new LP-BER proteins, RECQ1, ERCC1-XPF, and RPA, using the strategies suggested by the reviewer. The revised manuscript demonstrates the necessity of RECQ1 for BER with siRNA against RECQ1 (Figure 1E, Figure EV2A), with RECQ1 KO MEFs (Figure EV2E), and with stable cell lines expressing RECQ1 shRNA (Figure EV2F). Additionally, the stable RECQ1 KD cells were complemented with shRNA-resistant RECQ1, which restored repair and 5' patch formation, further confirming that RECQ1 is specifically required for 5' patch-mediated LP-BER (Figure EV2F).

To definitively show the critical involvement of ERCC1-XPF in LP-BER, repair assays were performed in wild-type and XPF mutant human fibroblasts (Figure 1H), which showed abrogation of repair and 5' patch formation in the XPF mutant HFs. Additionally, we present repair experiments performed in the XPF mutant HFs lentivirally transduced with wild type XPF (Staresincic et al, 2009) in which repair and 5' patch formation are restored (Figure EV3C). Additionally, we show that HCT116 cells with XPF KD by siRNA (Figure EV3D) lose repair capacity. The results of these experiments demonstrate that XPF is required for 5' patch-mediated LP-BER.

Finally, the revised manuscript demonstrates the necessity of RPA for BER with siRNA against RPA (siRPA32 #1) along with complementation with siRNA-resistant RPA (Figure 1G, Figure EV3A), in which repair and 5' patch formation were restored by complementation with siRNA-resistant RPA in RPA KD background. Additionally, in repair assays in HCT116 cells with a second, independent siRNA against RPA (siRPA32 #2), repair and 5' patch formation were abolished (Figure EV3B), confirming that RPA is critical for 5' patch-mediated LP-BER.

3. "Fig. 2B. The predicted 2.2 kb band is not visible. The studies with the 3' probe need to be explained in the Table shown in Fig. 2A."

Response: We thank the reviewer for noticing these issues with Figure 2. Regarding Figure 2B, there was a problem with the brightness of the image, especially once converted to PDF. In the revised manuscript we have replaced the panel (Figure 2B) with a gel image where the 2.2kb band is more readily visible. Additionally, we have added the 3' probe studies as a second table in Figure 2A.

5. "The authors present intriguing data that RECQ1 inhibits PARP1 activation. This effect is seen at low but not high NAD concentrations. There is no discussion of how this effect relates to physiological levels of NAD."

Response: We thank the reviewer for noting the missing critical information regarding physiological levels of NAD^+, and we have added the information in the revised manuscript (Please see page 12) as "gap formation did occur in the presence of physiologically relevant concentrations of NAD^+, which is found at a concentration of approximately 100 μM in the nucleus and cytoplasm (Figure 3C) (Koch-Nolte et al, 2011)_ENREF_53".

6. "The results of the pull down assays shown in Fig. 3E are not convincing and lack controls ie no DNA and non-biotinylated DNA. An EMSA may be more informative in terms of identifying DNA-protein complexes. "

Response: We thank the reviewer for his/her constructive critique of Figure 3E. In the revised manuscript we have replaced poor panels with better images from a repeat of the experiment (Figure 3E). Additionally, we have included controls (non-biotinylated nicked DNA and beads alone) to strengthen the results of the recruitment experiment.

7. "The authors provide evidence that RECQ1-dependent long patch BER is the major activity in cells. This consistent with other cell-based studies showing that long patch BER predominates over short patch BER. There is evidence for replication-associated BER that is assumed to occur via long patch. Is the RECQ1-dependent long patch BER pathway operational in non-diving or G1 cells?"

Response: We thank the reviewer for posing this relevant question regarding the role of 5' gap LP-BER in G1-arrested cells. We performed an experiment investigating BER in G1-arrested cells and have included this new data in the revised manuscript (Appendix Figure S3). We found that RECQ1-dependent LP-BER occurs in both proliferating and non-dividing or G1-arrested cells.

Reviewer #3:

1. "Despite the arguments presented, I'm still uncomfortable with referring loosely to this newly described process as BER (or LP-BER). Yes, it involves a DNA glycosylase and APE1, but otherwise deviates from what has been traditionally thought of as BER, both SP/SN-BER and LP-BER. As the authors point out, prior studies have reported a patch size of 6-7 nts for LP-BER (see Discussion), which is incompatible with their model. Thus, to distinguish the new process from the

previously described BER sub-pathways, the authors are encouraged to consider an alternative nomenclature, such as perhaps "5' gap LP-BER"."

Response: We thank the reviewer for his/her constructive critique of how we defined the newly discovered pathway. We agree with the argument that, at this point, distinguishing the new mechanism from the previously described BER sub-pathways is justified, and have referred to the mechanism as 5' gap LP-BER in the revised manuscript.

2. "It's hard (for me) to gauge how much of the total repair involves the new mechanism of 5' gap LP-BER versus the more traditional pathways. For example, can the authors estimate the percentage of etheno-A lesions that are processed via the new repair response (relative to classic BER mechanisms), and how is this percentage derived? Indeed, it's not totally clear what "percent repair" means, and as such, the authors are encouraged to spend a bit more time describing the details of the assay."

Response: We thank the reviewer for noting that the explanation of our assay was insufficient to explain what "percent repair" means in the figures. In fact, the reviewer is correct in surmising that a more clear explanation of the details of the assay execution and data interpretation will answer his/her first concern about "how much of the total repair involves the new mechanism of 5' gap LP-BER versus the more traditional pathways." We have included more details about the assay in the revised manuscript (Please see pages 4-5). Briefly, using the plasmid-based in-cell repair assay developed by our laboratory (Choudhury et al, 2008) and BER substrate DNA adducts (εA, 8oxoG, 5-OHU), we are able to quantify the percentage of repair events that occur via both 5' and 3' patch formation. Resolution of the parent lesion is denoted "Total repair" while resolution of a neighboring mismatch is used to detect patch formation and is denoted "patch formation." Mismatch resolution only occurs during patch formation in BER (instigated by the parent lesion) and not via the mismatch repair pathway (Appendix Figure S1D). Therefore, if mismatch resolution on the 5' side of the parent damage occurs in approximately 85-90% of repair events (as it does in our case), we can conclude that the 5' gap LP-BER constitutes 85-90% of total repair. Other possibly traditional pathways are then responsible of the remaining 10-15% of total repair.

3." I think it would be worthwhile for the authors to point out that, in addition to the location of the damage and relative position of the mismatch varying, the substrates in many instances possess a different sequence context as well, potentially influencing the outcome. There may also be sequence effects on pathway selection, possibly explaining the differences with prior studies. Perhaps some comment on this thought can be included."

Response: We thank the reviewer for raising this valid point about sequence context effects on repair, and we have included a comment on this issue in the Discussion section of the revised manuscript (Please see page 21).

4. "In some of the experiments, it's unclear what plasmid was used, making it difficult to interpret the results. See for example Figure 1E-H. While some assumptions can be made, the precise substrate should be stated explicitly in the legend on each occurrence."

Response: We thank the reviewer for this constructive criticism of the data presentation. We have identified the plasmid used for each experiment in the legend in the revised manuscript.

5. "It would be interesting to directly examine the participation of other 3' to 5' helicases, to determine the specificity of RECQ1 for the observed 5' gap-BER event. For example, have the other human RECQ paralogs (WRN, BLM) been evaluated using a knockdown approach?"

Response: We thank the reviewer for this suggestion, which will strengthen the conclusions of our study. We now have included repair data from knockdown of the other RecQ family members in the revised manuscript (Appendix Figure S6). Interestingly, we found that the involvement of other RecQ family helicases in BER supported our conclusions about the importance of PARP1 for 5' patch formation. It is true that none of the RecQ family helicases have been shown to play a role in BER specifically as helicases although they have been shown to play some indirect or non-helicase roles in BER by stimulating other proteins or affecting protein expression (Croteau et al, 2014). In fact, only RECQ5 has been shown to modulate expression of PARP1, as one study reported that KD

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of RECQ5 resulted in a significant decrease in PARP1 expression (Tadokoro et al, 2012). Indeed, we found that while repair of εA was independent of BLM, WRN, and RECQ4, it was somewhat dependent on RECQ5 (Appendix Figure S6). Moreover, 5' patch formation was completely abrogated by RECQ5 KD (Appendix Figure S6), supporting our finding that PARP1 is required for 5' patch formation. In the future it will certainly be interesting to investigate the apparently complex relationships among RECQ5, RECQ1, and PARP1 in LP-BER.

6. "Similarly, it would be better to more directly assess the involvement of MUS81 by using a knockdown strategy. Alternatively, the discussion of the MUS81 expression should be treated more cautiously, as the results are by no means definitive regarding its participation as an alternative enzyme in the process."

Response: We thank the reviewer for their valuable criticism regarding our interpretation of the Mus81 expression results as they relate to BER. We have provided a more cautious interpretation of the data in the revised manuscript (Please see page 10).

7. "The images of Figure 3E are not clear in several instances. The results would be strengthened by better images or by quantitation of the blots."

Response: We thank the reviewer for his/her constructive critique of Figure 3E. In the revised manuscript we have replaced poor panels with better images from a repeat of the experiment (Figure 3E). We have also provided quantitation of the blots (Figure 3G). Additionally, per a similar critique from reviewer #2, we have included controls (non-biotinylated nicked DNA and beads alone) to strengthen the results of the recruitment experiment.

8. "Given the degree of the effect of RECQ1 knockdown on 8oxoG repair and SSB accumulation (Fig 5), it seems surprising that knockdown has such a mild effect on cell survival. The authors should consider comparing the DNA damage levels and/or survival of OGG1-knockdown, RECQ1-knockdown and double-knockdown cells to get a better sense of the relationship between these two components."

Response: We thank the reviewer for his/her valuable suggestion. The revised manuscript provides better insight into the role of RECQ1 in repair of 8oxoG lesions in genomic DNA. After knocking down both OGG1 and RECQ1, SSB accumulation is significantly diminished, which is consistent with our hypothesis that the effect of RECQ1 KD alone on SSB accumulation is predominantly due to unresolved OGG1-generated BER intermediates (Figure 5H).

9. "What is known about the sensitivity of XPF or ERCC1-deficient cells to classic BER DNA-damaging agents, such as MMS or hydrogen peroxide? One would expect increased sensitivity as a function of its role in 5' gap LP-BER, and some discussion along these lines might be useful."

Response: We thank the reviewer for mentioning this valid point, which strengthens the conclusions of our study. We have included a discussion of evidence of the role of XPF in BER from the literature in the revised manuscript (Please see page 9). Briefly, XPF has been implicated in the repair of oxidative damage, in particular when 3' blocked ends are involved (Fisher et al, 2011). The aforementioned study found that both XPF-deficient Chinese hamster ovary (CHO) cells and ERCC1-deficient CHO cells are more sensitive to H_2O_2 than wild type CHO cells, which is consistent with our findings that ERCC1-XPF is playing a significant role in the resolution of oxidative DNA damage.

10. "The statement in the Discussion "which we have identified as RECQ1-dependent LP-BER" is inaccurate, as the authors (as far as I can tell) have not conducted studies using a uracil-containing substrate (OHU is not the same). Either the experiments need to be performed, or the sentence should be modified accordingly."

Response: We thank the reviewer for correcting the language used to compare the results of our study with the results of other studies. We have revised that section of the discussion to accurately reflect to what extent our study is consistent with comparable BER studies of other researchers (Please see page 22).

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Minor issues
1. "When printed, the text in many of the figures is very difficult to see, even with my 1.5X reading glasses."

Response: We apologize to the reviewer for the small text and have enlarged the font in the figures in the revised manuscript.

2. "Figure 1A. NA and ND need to be defined in the legend."

Response: We thank the reviewer for noting the missing information in the legend for Figure 1 and have provided definitions for NA and ND in the legend for Figure 1 in the revised manuscript.

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2nd Editorial Decision 02 January 2017

Thank you for submitting your revised manuscript on 5' gap formation in long-patch BER for our editorial consideration. Two of the original referees have now once more assessed the study in depth, and I am pleased to inform you that they both consider the key concerns raised during the first round of review satisfactorily addressed. Consequently, they now find the work suitable for publication in The EMBO Journal pending modification of some minor points, as detailed below, and I am therefore returning the study to you for a final round of revision in order to allow you to incorporate these last changes.

When resubmitting a re-revised manuscript, please also carefully address the following important editorial points:

- Please provide higher-resolution files for all main and EV figures, since both text and data in the currently provided PDFs appear (possibly due to compression) too pixelated.

- The close cropping, low resolution and contrast/brightness over-adjustments in many of the protein blot/gel data panels makes it difficult to properly assess the primary data behind them. Therefore, please include figure source data for all protein gels, blots and autoradiographs. We would ask for a single PDF/JPG/GIF file per figure comprising the original, uncropped and unprocessed scans of all such panels displayed in the respective figures. These should be labelled with the appropriate figure/panel number, and should have molecular weight markers; further annotation would clearly be useful but is not essential. These files can be uploaded upon resubmission selecting "Figure Source Data" as object type, and they would be linked as such to the respective figures in the online publication of your article.

REFEREE REPORTS

Referee #2:

In this study, the authors describe a novel subpathway of base excision repair in which a second incision occurs 5' to the incised damaged base generating a 5' gap. The authors have addressed all the issues raised in the previous critique. A particular strength of this study is how it integrates previous, sometimes contradictory published observations on base excision repair subpathways. For, example, the negative regulation of PARP1 by RECQ1 is an interesting and novel finding that explains some contradictory observations regarding the role of PARP1 in base excision repair and also provides mechanistic insight into the role of member of a RECQ DNA helicase family in excision repair. Overall this study constitutes a significant advance in our understanding of a key DNA repair pathway and insights into the function of a family of DNA helicases linked with human disease.

Minor comment
Fig. 3E; symbols indicating reaction components need to be aligned
pg 21 Second sentence, "It should...." needs to be split into two sentences.

Referee #3:

The manuscript here presents evidence for a novel mechanism of BER, termed 5' gap-mediated long-patch BER, which engages the 3'-5' RECQ1 helicase, the single-stranded DNA binding protein RPA and the ERCC1/XPF nuclease complex. Selection of this process appears to be dictated by PARP1 activity at the nick intermediate, which is specifically regulated via its interaction with RECQ1. The authors have adequately responded to my prior concerns, and I only have a few remaining minor issues that should be addressed.

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Minor
p. 6. APE should be APE1. Similarly, POLbeta (Greek letter), not POLB.
p. 12. Figure 3B, lane 9 should be Figure 3D. Once corrected, 3D now appears before 3C.
Figure 3B, lane 8. The text on p. 13 suggests that the reaction includes a catalytically inactive RECQ1 protein. However, the figure image suggests no RECQ1. "-" is different from a mutant protein. Please clarify.
p. 13. The following statement is inaccurate: "RECQ1 was only significantly recruited to the substrate in the presence of PARP1 or RPA." Certainly there is RECQ1 bound to the DNA in the absence of PARP1 or RPA (see Figure 3). Please correct sentence.

2nd Revision - authors' response 07 March 2017

Responses to the editor and reviewers:

Editor
- Higher resolution files for all main and EV figures.
- Source files for all images

Response: We have addressed the points (suggested by the editor) per requirement for the manuscript revision.

Reviewer 2

Minor comments
1. Figure 3E: Align symbols indicating reaction components
2. Page 21 Second sentence, "It should...." needs to be split into two sentences.

Response: We thank the reviewer for noting these 2 issues to improve our manuscript. We have incorporated the suggested changes accordingly in the re-revised manuscript.

Reviewer 3

Minor comments
1. p. 6. APE should be APE1. Similarly, POLbeta (Greek letter), not POLB.

4. p. 13. The following statement is inaccurate: "RECQ1 was only significantly recruited to the substrate in the presence of PARP1 or RPA." Certainly there is RECQ1 bound to the DNA in the absence of PARP1 or RPA (see Figure 3). Please correct sentence.

Response: We thank the reviewer for noting these 2 issues to improve our manuscript. We have incorporated the suggested changes accordingly in the re-revised manuscript.

2. p. 12. Figure 3B, lane 9 should be Figure 3D. Once corrected, 3D now appears before 3C.

Response: We apologize for the confusing statement in the manuscript. We have now corrected the text to reflect the actual experimental condition used in Figure 3B, lane 9 that we added PARP1, which was, in fact, previously PARylated before its addition in the gap formation reaction. In Figure 3D, PARylated PARP is shown as a reaction product.

3. Figure 3B, lane 8. The text on p. 13 suggests that the reaction includes a catalytically inactive RECQ1 protein. However, the figure image suggests no RECQ1. "-" is different from a mutant protein. Please clarify.

Response: We apologize for the confusion. In the current re-revised version, the addition of catalytically inactive RECQ1 for lane 8 of Figure 3B is clearly denoted as “helicase dead RECQ1”.

3rd Editorial Decision 09 March 2017

Thank you for submitting your final revised manuscript for our consideration. I am pleased to inform you that we have now accepted it for publication in The EMBO Journal.

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YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ↓

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Rabindra roy
Journal Submitted to: EMBO J
Manuscript Number: EMBOJ-2016-94920R

Reporting Checklist For Life Sciences Articles (Rev. July 2015)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures

1. Data

The data shown in figures should satisfy the following conditions:

- ➔ the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ➔ figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
- ➔ graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
- ➔ if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
- ➔ Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- ➔ a specification of the experimental system investigated (eg cell line, species name).
- ➔ the assay(s) and method(s) used to carry out the reported observations and measurements
- ➔ an explicit mention of the biological and chemical entity(ies) that are being measured.
- ➔ an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- ➔ the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- ➔ a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- ➔ a statement of how many times the experiment shown was independently replicated in the laboratory.
- ➔ definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

Please ensure that the answers to the following questions are reported in the manuscript itself. We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

In the pink boxes below, provide the page number(s) of the manuscript draft or figure legend(s) where the information can be located. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable).

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B- Statistics and general methods

Please fill out these boxes ↓ (Do not worry if you cannot see all your text once you press return)

Question	Answer
1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	NA
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	NA
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	NA
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	NA
For animal studies, include a statement about randomization even if no randomization was used.	NA
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	NA
4.b. For animal studies, include a statement about blinding even if no blinding was done	NA
5. For every figure, are statistical tests justified as appropriate?	Yes
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	NA
Is there an estimate of variation within each group of data?	NA
Is the variance similar between the groups that are being statistically compared?	NA

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	We have authenticated all the antibodies used in this study using siRNA-mediated knockdown cells and positive and negative controls. Details of antibody sources are found in Appendix, page #17.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	We have authenticated all cell lines such as HCT-116, HeLa proposed in this study using short tandem repeat (STR) analysis. Results of these assays were compared to the American Type Culture Collection (ATCC). Also we routinely tested for mycoplasma contaminations for all the cells under this study.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

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9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	NA
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	NA

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	NA
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	NA
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	NA
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	NA
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	NA
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17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	NA

F- Data Accessibility

18. Provide accession codes for deposited data. See author guidelines, under 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	NA
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	NA
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	NA
21. As far as possible, primary and referenced data should be formally cited in a Data Availability section. Please state whether you have included this section. Examples: **Primary Data** Wetmore KM, Deutschbauer AM, Price MN, Arkin AP (2012). Comparison of gene expression and mutant fitness in Shewanella oneidensis MR-1. Gene Expression Omnibus GSE39462 **Referenced Data** Huang J, Brown AF, Lei M (2012). Crystal structure of the TRBD domain of TERT and the CR4/5 of TR. Protein Data Bank 4O26 AP-MS analysis of human histone deacetylase interactions in CEM-T cells (2013). PRIDE PXD000208	NA
22. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	NA

G- Dual use research of concern

23. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	NA