MYST Protein Acetyltransferase Activity Requires Active Site Lysine Autoacetylation

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Thank you for submitting your manuscript for consideration by the EMBO Journal. Three referees have now evaluated your study and I enclose their reports below. While the referees find that the current study is potentially interesting they unfortunately raise a number of important issues that preclude publication in the EMBO Journal.

I hope that you understand if I do not go though each individual criticism in this letter, but two of the referees raise a number of important concerns regarding the mechanism of activation by autoacetylation. I have discussed the reports with these referees and they are in agreement that given the known role of autoacetylation in regulating hMOF further mechanistic understanding of its role in regulating HAT activity is required. This includes structural information demonstrating the mode of substrate binding by the MYST proteins and also the structural basis of inactivation in the Lys to Arg mutant. These are also required to potentially reveal why the effects of a number of mutations currently contradict the current model. Furthermore, a more comprehensive biophysical characterisation of the peptide binding assays is also requested. I realise that asking for two further crystal structures, in complex with substrate and a Lys to Arg mutant is a large and significant additional amount of work to ask for, however, it is clear that we are unable to proceed with publication without detailed mechanistic insight into the regulatory role of autoacetylation. Importantly, it is clear from the reports that addressing these concerns would take a significant amount of time and longer than the three months that the EMBO Journal allows for a period of revision. Therefore, since we can only afford to continue handling of papers that receive enthusiastic support from at least a majority of referees upon initial review, I am afraid, I see little choice but to
come to the conclusion that we cannot offer to publish this study.

However, as I mentioned the majority of referees find the study to be potentially interesting. Therefore, if you are able to extend the study experimentally as suggested we would be willing to look at the manuscript once more. I would like to stress that if you were to follow this option I would try to get the same referees to look at the study once more, however depending on their availability this manuscript may be viewed as a new submission rather than a revised manuscript; this could potentially entail the selection of new referee(s) and may involve a new round of peer review. If you decide to thoroughly expand the manuscript and submit an improved version to the EMBO Journal, please make sure to mention the initial manuscript number to allow efficient handling. At this stage of analysis, though, I am sorry to have to disappoint you.

Thank you in any case for the opportunity to consider your manuscript. I am sorry we cannot be more positive on this occasion, but we hope nevertheless that you will find our referees' comments helpful. We also hope that this negative decision does not prevent you from considering our journal for publication of your future studies.

Yours sincerely

Editor
The EMBO Journal

REFEREE REPORTS

Referee #1

Review: "MYST Protein Acetyltransferase Activity Requires Active Site Lysine Autoacetylation"

In this work Yuan and colleagues report the crystal structures of yeast Esa1 and human MOF proteins. They found both enzymes to be autoacetylated on a conserved lysine residue and subsequently confirmed this finding by mass spectrometry and showed its importance for the biological function of both enzymes.

While phosphorylation and its effects on protein function have been intensely studied for a long time now, acetylation and other post-translational modifications of proteins (other than histones) have received much less attention and constitute an emerging and fascinating field of study. The experiments described in this manuscript were well designed and executed, and the results are compelling.

As the authors mention in their discussion, SIRT1 has been shown to deacetylate hMOF and affects hMOF recruitment in vivo. Does Sir2 also deacetylate yEsa1?

Referee #2

In their manuscript, Yuan et al. show that MYST protein lysine acetyltransferases require active site auto-acetylation at a strictly conserved lysine residue. The position of a partially non-acetylated lysine residue in hMOF suggests that the non-acetylated lysine blocks access of the substrate to the active site. Additional experiments demonstrate the importance of auto-acetylation for MYST protein activity in vitro and in vivo. Overall, the study convincingly demonstrates the importance of auto-acetylation of a conserved lysine (yEsa1-K262, hMOF-K274) for the acetylation activity of MYST protein lysine acetyltransferases.

Auto-acetylation has been already described in the HAT domain of p300/CBP and Rtt109, although the activation mechanisms are probably different. In addition, Kadlec et al., (NSMB 2011) also reported acetylation of hMOF-K274 and showed that mutating this residue into alanine abolished its catalytic activity. As such the results of Yuan et al. are therefore not novel and unexpected.

A major weakness of the manuscript is the fact that the mechanism by which auto-acetylation
activates MYST protein lysine acetyltransferases is not convincingly explained. The authors argue that in its unacetylated form residue yEsa1-K262 (hMOF-K274) would block the access of the substrate to be acetylated. However, this explanation is rather speculative. There is no structure of a MYST protein/substrate complex known to support this claim and the authors do not show the access path or cavity that need to be blocked. The explanation provided by the authors also seems to contradict the results from Kadlec et al.: Removing the side chain of K274 by mutating this residue into an alanine should allow the substrate to better access the active site and the enzyme should be more active, whereas the K274A mutant shows strongly reduced acetylation activity. It therefore is possible that auto-acetylation activates through alternative or additional mechanisms.

The authors need to better elucidate the activation mechanism by auto-acetylation. For example at present, the authors only use quenching of tryptophane fluorescence to monitor binding of a H4 peptide to the active site. A more quantitative analysis of peptide binding (for example by ITC) and HAT activity of wild type and mutant yEsa1 and hMOF proteins (including the K274A mutant) might give additional insight into the role of the acetylated lysine in MYST protein activation.

Minor points: Figure 1F is missing.

Referee #3

In this manuscript Marmorstein and colleagues explore the possibility that MYST protein HATs are regulated by Lys auto-acetylation of a catalytic site residue. They demonstrated by crystallography and mass spectrometry that Lys262 of yeast Esa1 and the equivalent Lys 274 of human MOF are acetylated. The acetylated Lys is highly conserved and forms contacts with conserved residues at the catalytic site. In the human protein there is a population of unmodified Lys274 that occupies the cognate substrate-binding site. The authors also demonstrate using bacterially expressed protein that acetylation occurs in cis (based on first order kinetic rate constants) and is therefore an auto-acetylation process. Mutation of Lys to Arg abolishes catalytic activity in Esa1, MOF and Sas2. Arg presumably mimics the un-acetylated Lys residue. Using a fluorescence-based binding assay, the authors show that whereas the wild type MOF binds peptides, the Arg mutant is unable to. Finally, the authors demonstrate that Esa1, MOF are acetylated on Lys 262 and Lys 274 in vivo and that mutation of the Lys to Arg abolishes in vivo activity of Esa1, MOF and Sas2. Given these results the authors suggest a model that in MYST HATs, catalytic site Lys auto-acetylation is a control mechanism analogous to protein phosphorylation.

Overall this is an interesting manuscript that is clearly written and illustrated. While many of the experiments are carefully performed, and the evidence that Lys262 and Lys 274 of Esa1 and MOF, respectively are acetylated, the data that Lys acetylation actually regulates the catalytic activity of the MYST proteins is not so definitive. The key data that Lys acetylation is regulatory is that in the MOF crystal structure, the unmodified Lys side chain occupies the cognate Lys substrate-binding site. To provide more support for their model, the authors should perform additional experiments. Also some of the authors' findings were reported by Kadlec et al., 2011.

1. Their model that acetylation of the catalytic Lys activates MYST seems based on the finding that the Lys to Arg mutant is inactive. The structural rationale for this wasn't explained. Is this because Arg adopts the conformation of the unacetylated Lys residue, thus blocking activity? If so, a Lys to Ala mutant would be expected to be active. This should have been tested. The data in Kadlec et al., 2011 shows that in MOF the K274A mutant is inactive.
2. For Esa1, a Lys to Gln mutant is functionally active (Fig. 3). However, Lys to Gln and Met mutants of Sas2 are not (Fig. 4). This seems contradictory and should be explained. Was the activity of the Esa1 Lys to Gln mutant tested in vitro?
3. Related to the point 1, a structure of the Lys to Arg mutant would be of interest.
4. The notion that the Lys to Arg mutant blocks cognate peptide binding was demonstrated in the fluorescence-binding assay (Fig. 2). However, the conclusions of this assay could be strengthened, first by testing a Lys to Gln mutant (which presumably should bind peptide similarly to wild type) and second the de-acetylated MOF protein should be tested in the presence or absence of CoA. It would then be interesting to test the affects of addition of acetyl-CoA. According to their model, peptide binding should be restored.
5. Can the authors speculate why auto-acetylation doesn't require Glu338/350?
6. To definitively confirm auto-acetylation, can inactive mutants of Esa1 or MOF be generated?
7. The notion that Lys262/274 is auto-acetylated would require that the Lys Nz atom is sufficiently close to the acetyl group of acetyl-CoA for nucleophilic attack of the Lys onto the cofactor. Was this modelled?
8. Fig. 1F (mass spectrum) is missing.

Reviewer 1

1. **As the authors mention in their discussion, SIRT1 has been shown to deacetylate hMOF and affects hMOF recruitment in vivo. Does Sir2 also deacetylate yEsa1?**

Although SIRT1 has been implicated to deactylate hMOF by Liang and coworkers (Lu et al. 2011, Cell Res., 21, pp 1182-) we have been unable to detect hMOF deacetylation in the laboratory using recombinant SIRT1. This could imply that other cofactors are required for SIRT1-mediated hMOF deacetylation in cells. To our knowledge, there is no evidence for Sir2-mediated deacetylation of yEsa1 in cells, although we are able to deacetylate yEsa1 with yHst2 in vitro, although not to completion. Although we now elaborate on these in vitro findings in the Discussion section of the revised manuscript, we feel that additional experiments that address the regulatory role of hMOF or yEsa1 deacetylation in cells goes beyond the scope of this manuscript.

Reviewer 2

1. **Auto-acetylation has been already described in the HAT domain of p300/CBP and Rtt109, although the activation mechanisms are probably different. In addition, Kadlec et al., (NSMB 2011) also reported acetylation of hMOF-K274 and showed that mutating this residue into alanine abolished its catalytic activity. As such the results of Yuan et al. are therefore not novel and unexpected.**

We are certainly aware that previous reports have found that other families of HAT proteins are also autoacetylated and that the hMOF member of the MYST proteins is also acetylated at the same lysine that we have identified. However, we believe that our findings are highly significant and novel for the following reasons. (1) Our study presents the first molecular and mechanistic insights into how autoacetylation mediates cognate protein substrate acetyltransferase activity in the MYST family. Specifically, we show that autoacetylation promotes protein substrate binding. This draws striking similarity to how protein kinases are activated by autophosphorylation and this mechanistic correlation has never been previously demonstrated. (2) Our study demonstrates that the MYST acetylation modification is indeed autoacetylation as opposed to acetylation by another protein. To our knowledge, this has not previously been demonstrated for the MYST proteins. (3) While other studies have demonstrated acetylation of selected HAT proteins, we show that this phenomenon occurs with three different MYST protein acetyltransferases across species on a strictly conserved lysine residue. This strongly argues that this posttranslational modification is conserved throughout the entire MYST protein family. Other studies do not present evidence to support this conclusion. (4) We demonstrate that this MYST autoacetylation activity is required for the biological properties of three different MYST proteins showing, for the first time, the biological importance of this posttranslational modification to the function of the yeast Esa1 and Sas2 MYST proteins. While other studies have also demonstrated the functional importance of human MOF autoacetylation, the functional importance of yEsa1 and ySas2 autoacetylation had not previously been characterized. Taken together, we feel that there are several significant and novel aspects of our study that would be of special interest to readers of EMBO Journal.

2. **A major weakness of the manuscript is the fact that the mechanism by which auto-acetylation activates MYST protein lysine acetyltransferases is not convincingly explained. The authors argue that in its unacetylated form residue yEsa1-K262 (hMOF-K274) would block the access of the substrate to be acetylated. However, this explanation is rather speculative. There is no structure of a
MYST protein/substrate complex known to support this claim and the authors do not show the access path or cavity that need to be blocked. The explanation provided by the authors also seems to contradict the results from Kadlec et al.: Removing the side chain of K274 by mutating this residue into an alanine should allow the substrate to better access the active site and the enzyme should be more active, whereas the K274A mutant shows strongly reduced acetylation activity. It therefore is possible that auto-acetylation activates through alternative or additional mechanisms.

To address this point, we now report in the revised manuscript an additional structure of yEsa1 bound to an H4K16CoA bisubstrate inhibitor (revised Figures 1A and 1B). This structure allows us to clearly define the position of the cognate substrate lysine in the active site of the enzyme and reveals that acetylation of yEsa1-K262 nicely accommodates binding of the cognate substrate lysine. Moreover, superposition of this structure with the hMOF structure containing K274 and K274Ac clearly shows that K274 or a modeled mutation to arginine or even an alanine would sterically occlude cognate substrate lysine binding (revised Figure 1E). In the revised manuscript, we also now show that mutation of the autoacetylated active site lysine in hMOF, yEsa1 and ySas2 to alanine also inhibits cognate protein substrate acetylation by these enzymes (revised Figure S3) and cognate protein substrate binding by hMOF (revised Figure 2C). Taking the structural and mutational data together, this data further supports the conclusion that active site lysine autoacetylation is important for MYST cognate protein substrate binding and acetylation. However, we do acknowledge that this autoacetylation might have other roles. We now also elaborate on this in the Discussion section of the revised manuscript.

3. The authors need to better elucidate the activation mechanism by auto-acetylation. For example at present, the authors only use quenching of tryptophane fluorescence to monitor binding of a H4 peptide to the active site. A more quantitative analysis of peptide binding (for example by ITC) and HAT activity of wild type and mutant yEsa1 and hMOF proteins (including the K274A mutant) might give additional insight into the role of the acetylated lysine in MYST protein activation.

We have tried to carry out MYST protein/protein substrate binding studies using ITC (in the presence or absence of CoA or AcCoA using catalytically defective MYST proteins) but unfortunately could not detect any heat release (or absorbed) upon titration of the peptide substrate. We are not sure why this is the case but note that, to our knowledge, no one has reported peptide binding to acetyltransferase enzymes using ITC. In the revised manuscript, we did, however, use the quenching of tryptophane fluorescence assay to monitor the binding of the H4 peptide to the hMOF K274A mutant and demonstrated a similar defective in binding the hMOF K274R mutant (revised Figure 2C). This data supports the conclusion that active site lysine autoacetylation is important for MYST cognate protein substrate binding.

4. Figure 1F is missing.

We apologize for this oversight and now include Figure 1F in the revised manuscript.

Reviewer 3

1. Their model that acetylation of the catalytic Lys activates MYST seems based on the finding that the Lys to Arg mutant is inactive. The structural rationale for this wasn't explained. Is this because Arg adopts the conformation of the unacetylated Lys residue, thus blocking activity? If so, a Lys to Ala mutant would be expected to be active. This should have been tested. The data in Kadlec et al., 2011 shows that in MOF the K274A mutant is inactive.

In the revised manuscript, we now more clearly describe our model that mutation of the acetylated lysine residue to either arginine or alanine adopt the “out” conformation of the unacetylated lysine because our structure suggests that the “in” conformation of the acetylated lysine cannot be easily accommodated by these amino acid substitutions. To support this conclusion experimentally, we now present additional mutational data in the revised manuscript. Specifically, we now show that mutation of hMOF-K274, yEsa1-K262 and ySas2-K168 to either arginine or alanine is also defective in catalysis (Figure 2B and revised Figure S3) and we show that hMOF-K274 mutation to arginine or alanine is defective in histone substrate binding (revised Figure 2C). Modeling of either of these mutations in place of K274 in the unacylated form would form a steric clash with the
cognate lysine substrate, as observed in the yEsa1 H4K16CoA bisubstrate complex (revised Figure 2E), consistent with the inability of these mutations to support cognate substrate acetylation. This point is elaborated in the Discussion section of the revised manuscript.

2. For Esa1, a Lys to Gln mutant is functionally active (Fig. 3). However, Lys to Gln and Met mutants of Sas2 are not (Fig. 4). This seems contradictory and should be explained. Was the activity of the Esa1 Lys to Gln mutant tested in vitro?

To address the reviewer’s concern, we prepared the hMOF-K274Q, yEsa1-K262Q and ySas2-E168Q mutants (as possible acetyl-lysine mimics) and assayed their catalytic activity in vitro (revised Figure S3). Consistent with the in vivo data on yEsa1 and ySas2, yEsa1-K262Q shows significant activity (about 25% of wild type) while ySas2-E168Q activity cannot be detected. In vitro, hMOF-K274Q also does not show activity (revised Figure S3) although we did not assay this mutant in vivo. These results suggest that there may be some differences in the mechanism of how active site lysine acetylation promotes enzyme activity within the proteins. This point is elaborated in the Discussion section of the revised manuscript.

3. Related to the point 1, a structure of the Lys to Arg mutant would be of interest.

We agree with the reviewer that this structure would be of interest, however, we have tried quite hard but were unable to crystallize this mutant as well as the alanine mutant of hMOF and yEsa1. We were, however, able to crystallize yEsa1 bound to a H4K16CoA bisubstrate inhibitor (revised Figures 1A and 1B), and this structure, together with the structure of hMOF in both the K274 acetylated and unacetylated forms (revised Figure 1E) make a stronger case that a Lys to Arg substitution would disrupt cognate lysine substrate binding as described in response to point 1 above.

4. The notion that the Lys to Arg mutant blocks cognate peptide binding was demonstrated in the fluorescence-binding assay (Fig. 2). However, the conclusions of this assay could be strengthened, first by testing a Lys to Gln mutant (which presumably should bind peptide similarly to wild type) and second the deacetylated MOF protein should be tested in the presence or absence of CoA. It would then be interesting to test the affects of addition of acetyl-CoA. According to their model, peptide binding should be restored.

We attempted the experiments suggested by the reviewer but could not obtain definitive results because of technical limitations of the experiments. Specifically, we were unable to prepare homogeneously deactylated yEsa1 or hMOF (using either recombinant yeast Hst2 or human SIRT1) for the fluorescence experiments so could not carry out reliable fluorescence quenching experiments with the deactylated and reacetylated proteins. In addition, we attempted to carry out fluorescence quenching experiments with yEsa1 but found that the level of fluorescence quenching was very low when H4 peptide was titrated into the wild-type enzyme. We therefore could not reliable compare the fluorescence quenching of yEsa1 mutants. Nonetheless, we believe that the additional biochemical experiments described under the responses to points 1 and 2 above, as well as the additional structure of yEsa1 bound to the H4K16CoA bisubstrate inhibitor and its comparison with hMOF in the K274 acetylated and deacetylated form significantly strengthen the argument that active site lysine acetylation of the MYST proteins contributes to cognate protein substrate binding and acetylation.

5. Can the authors speculate why auto-acetylation doesn’t require Glu338/350?

To address the request by the reviewer, we have added a short paragraph to the Discussion section of the revised manuscript that speculates about why yEsa1 autoacetylation (and probably autoacetylation of other MYST proteins) does not appear to be as dependent as cognate protein substrate acetylation on the catalytic Glu338 general base residue. Specifically, we argue that although there are many factors that influence enzyme catalytic efficiency, one particularly important factor is the appropriate templating of substrates in appropriate geometry for catalysis. Indeed, if one compares the catalytic mechanism of the four HAT families, Gcn5/PCAF, MYST, p300 and Rtt109, one finds that although they employ different catalytic residues and mechanisms for catalysis, they contain a structurally conserved core region that plays a conserved templating function. Since autoacetylation occurs intramolecular, it has a templating advantage over
intermolecular cognate protein acetylation since the effective substrate concentration is higher and the activation energy for appropriate lysine substrate orientation is likely lower. This is consistent with the about 15-fold higher apparent $K_{m}$ for cognate protein substrate acetylation (154 mM, reported in Yan et al. 2002, *NSMB*, 9, pp 862-) over autoacetylation (9.4 mM, Figure S1E). As a consequence of this, we propose that autoacetylation is less dependent on the contribution of the Esa1-E338 general base than cognate protein substrate acetylation, although the possibility that an alternative pathway or mechanism for autoacetylation cannot be ruled out.

6. To definitively confirm auto-acetylation, can inactive mutants of Esa1 or MOF be generated?

We were unable to prepare yEsa1, hMOF or ySas2 mutants that were defective in autoacetylation. This is consistent with the observation that autoacetylation is less dependant on catalytic residues that are important for cognate protein substrate acetylation, as discussed in our response to point 5 above. Nonetheless, we believe that taking our structural, biochemical and structural data together makes a strong case for autoacetylation.

7. The notion that Lys262/274 is auto-acetylated would require that the Lys Nz atom is sufficiently close to the acetyl group of acetyl-CoA for nucleophilic attack of the Lys onto the cofactor. Was this modelled?

To address the concern of the reviewer, we now note in the Results section entitled “Active site acetylation by MYST proteins occurs by autoacetylation” of the revised manuscript that autoacetylation in cis is consistent with the structural observation that the autoacetylated lysine is located within the enzyme active site and about 5 Å away from the catalytic cysteine residue (C304 in yEsa1 and C316 in hMOF) that could transfer the acetyl group via a ping-pong catalytic mechanism (Yan et al. 2002, *NSMB*, 9, pp 862-) and about 7 Å from the CoA sulfur atom that could transfer the acetyl group via a ternary complex mechanism (Berndsen et al. 2007 *Biochemistry*, 46, pp 623-). Therefore, a relatively minor movement of the acetylated lysine side chain (for example a change of rotamer) and/or acetyl donors would easily accommodate autoacetylation in cis.

8. Fig. 1F (mass spectrum) is missing.

We apologize for this oversight and now include Figure 1F in the revised manuscript.

2nd Editorial Decision 19 September 2011

Thank you for submitting a revised version of your manuscript. It has now been re-evaluated by two of the original referees who find that the study has been significantly strengthened. I am happy to accept the manuscript for publication in The EMBO Journal. You will receive the official acceptance letter in the next day or so.

Thank you and best wishes,

Editor
The EMBO Journal

Referee #2

In the revised version, the authors have addressed most issues raised by the referees. In particular, the additional yEsa1:H4K16Coa inhibitor structure has significantly strengthened the manuscript. I now recommend publication without further delay.
Referee #3

The authors have addressed many of my concerns adequately. While an effort was made to address others, technical difficulties prevented this.

The findings are of sufficient interest and importance for the manuscript to be worthy of publication in EMBO J.