nature portfolio

Peer Review File

PKA plays a conserved role in regulating gene expression and metabolic adaptation by phosphorylating Rpd3/HDAC1

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The manuscript authored by Wenjing Dai et al. deals with the phosphorylation of yeast Rpd3L consequent to shift to nutritional status in the presence of sucrose as energy source. The Authors aimed to unveil how yeast cells sense extracellular sucrose signals and respond with alterations of histone modifying enzymes. They identified the GPCR Gpr1 as the nutrient sensor of sucrose with high affinity binding for this disaccharide in yeast. Furthermore, they described a signaling pathway that directly connect nutrient sensing with chromatin modifications. In detail, they found that Tpk2-catalyzed Rpd3 phosphorylation impairs the deacetylase activity of Rpd3 and diminished Ada3 deacetylation within SAGA. This effect is mediated by the direct interaction between Rpd3L specific subunit Ash1 and SAGA subunit Ubp8, thus connecting extracellular sucrose with repression of Rpd3L and auto-acetylation of SAGA. In the final series of experiments the Authors found that PKA phosphorylates the class I histone deacetylase (HDAC) HDAC1 in mammalian cells, and by reducing its deacetylase activity it increases histone acetylation and enhances TCA cycle. Along this line, the Authors hypothesized that PKA could promote tumorigenesis by phosphorylating and inhibiting HDAC1, since it has been reported that PKA promotes hepatocellular carcinoma invasion and metastasis by phosphorylating CIP4.
The Authors performed a very detailed analysis of the biochemical mechanisms of Rpd3/HDAC1 regulation by nutrients, which allowed to identify a signaling pathway linking extracellular nutrients to epigenome modifications and metabolic regulation.

I have the following comments to the manuscript:

1. In many figure panels the Authors used two-tailed t-tests which does not seem appropriate. For instance, in supplementary figure 2d the Authors report “two-tailed t-tests were used for statistical analysis” in the legend. However, in this case there are more than two groups in the experiment, therefore a two-way ANOVA would seem more appropriate as the variables in this experiment are the treatment with YP, YP+glucose, YP+sucrose and the genotype (wt, pde1mutant, pde2mutant). Statistical analysis should be reviewed for data reported in this graph. Also, the comparisons should be clearly stated in the figure legend (e.g., **, $p<0.01$; ***, $p<0.001$ refer to what comparisons?). Please, review the statistical analyses in all figures and apply the appropriate test accordingly.
2. In figure 2i the Authors show that loss of Gpr1 abrogated sucrose-induced increase of cAMP. What happens to cAMP levels if Gpa2 is mutated? In the present version of the manuscript the Authors only show that loss of Gpa2 attenuated sucrose-induced Ada3-K14ac and Ada3-K182ac. The Authors should also show whether loss of Gpa2 blunts the effect of sucrose on cAMP levels.
3. For the sake of clarity: it seems that Rpd3-2SA be a double mutant on both S50 and S354 amino acid residues. This should be more clearly explained. I could not find any details about this mutant in the “Methods” section. Likewise, for Ash1-2SA mutant. In general, there is no description of how these mutants have been made in the “Methods” section.
4. Figure 3f: these images must be quantified. It is not clear from the immunofluorescence images whether sucrose increased phosphorylation of Rpd3 on S50 and S354 in wt cells when compared to cells treated with glucose.
5. Figure 4b reporting immunofluorescence analysis of Ada3 acetylation: the images must be quantified.
6. Again, I have reservation about the statistical analyses in the figures 4a and 4c: the Authors performed two-tailed t-tests. However, the variables in these two graphs are two (i.e., treatment glucose vs. sucrose, and wt vs. mutant), therefore a two-way ANOVA should be applied. In addition, it is not clear to what comparisons the statistically significant differences refer according to the figure legend.
7. The western blot in supplementary figure 4b, showing that the mutation of Rpd3-2SD rescued the reduced Ada3-K14ac

and Ada3-254 K182ac in tpk2Δ mutant, should be quantified.
8. In experiments reporting kinetic analysis of protein phosphorylation or acetylation (e.g. figure 1b, 1c, 1e, 2f, 3i, 4d, 4e, 4f etc. and in supplementary figures as well) the Authors added P values in the graphs, but I could not find what these P values refer to. Is it by perhaps referred to AUCs? Please, clearly specify them in all figure legends.
9. The western blots in supplementary figure 4e and 4f should be quantified.
10. There are several western blots of co-IP experiments that should be quantified. The Authors make statements about enhanced or reduced interactions depending on mutant or culture conditions. For instance, in figure 6f they claim that "In vivo Co-IP showed that mutation of Ash1-2SA enhanced the intracellular interaction with Ubp8 and Ada3 within SAGA and mutation of Ash1-2SD reduced its interaction with Ubp8 and Ada3 within SAGA". These differences are often subtle and hard to appreciate; quantification should be performed to consolidate the statements, which otherwise would be somewhat questionable. Another example is in figure 7a and supplementary figure 7a, where the Authors state that loss of Tpk2 reduced Ada3 dimerization. Please, note that there are other similar cases in the manuscript.
11. Pg 17, lines 380-381: it seems that the Authors indicated the wrong figure in the text. It should be supplementary figure 7c and not figure 7c, when presenting the data relative to global levels of H3K9ac and H3K14ac, which gradually increased when cells were shifted from YP medium to YP + 2% glucose medium or to YP + 2% sucrose medium. In fact, in figure 7c there is no switch from YP medium to YP medium + glucose and YP medium + sucrose. Again, here the quantification of the western blot images in supplementary figure 7c should be added.
12. In figure 7d and in supplementary figure 7d the Authors show that the sucrose-dependent-induction of SUC2 is reduced with Ada3-3KR, Rpd3-2SA, TpkaΔ and Gpr1Δ mutants. However, although the induction is reduced with mutants, sucrose can still induce SUC2 transcripts quite significantly. Why? Is there another mechanism underlying the sucrose-mediated induction of SUC2? Also, the statistical analysis in supplementary figure 7b and supplementary figure 7d was performed with two-tailed t-tests, however, since there are more variables, two-way ANOVA should be applied. Likewise in figure 7c and in figure 7d.
13. PKA-induced phosphorylation of Rpd3/HDAC1 represses Rpd3/HDAC1 activity and leads to increased mRNA levels of genes encoding enzymes of TCA cycle in yeast and in HepG2 cells. However, the Authors only show hyperacetylation of nucleosomes on SUC2 promoter in yeast while they do not show whether the hyperacetylation occurs to the promoter/enhancer of genes of the TCA cycle in yeast treated with sucrose and in HepG2 cells treated with cAMP. This is a significant piece of information that is missing in the manuscript. It would be important to show that promoter/enhancer regions are hyperacetylated at least for some genes involved in TCA cycle in yeast and in HepG2 cells.
14. In line with the previous comment, hyperacetylation of promoter/enhancer regions requires acetyl-CoA as the acetyl donor for histone acetylation of target genes. ATP-citrate lyase (ACLY) is the enzyme providing acetyl-CoA for histone acetylation and has been demonstrated to localize also in the nucleus, where it cleaves citrate to acetyl-CoA and oxaloacetate in mammalian cells (SCIENCE, 22 May 2009, Vol 324, Issue 5930, pp. 1076-1080 DOI: 10.1126/science.1164097). The Authors should test whether treatment with PKA activator increases the amount of ACLY in nuclei of HepG2 cells. This could be easily assessed by immunofluorescence microscopy.

Minor points:
1. Supplementary figure 7i: it is not clear what "1N" and "2N" stand for. Please, specify in the figure legend to help the reader.
2. The abbreviations of genes tested in figure 7 and 8 (and the corresponding supplementary figures) should be spelled out.
3. In supplementary figure 8g HepG2 cells were treated with cAMP. What is the chemical compound the Authors used? Is it a stable analog (e.g., 8-Br-cAMP)? Please, specify it in the methods section as well as in the figure legend.

Reviewer #2

(Remarks to the Author)
In this study, Dai et al discovered that PKA can phosphorylate Rpd3/HDAC1 and regulate the expression of metabolic genes, which is crucial in mediating the metabolic adaptation of yeast, especially when cells are cultured in medium with sucrose as the sole carbon source. In general, the findings are interesting, and the data are solid. I have some comments regarding the functional contribution of this pathway to the cell fate change and its conservation in mammals.

1. What is the functional contribution of this Tpk2-Rpd3L pathway to the yeast cell fate change? Since this pathway is considered important for the metabolic adaptation of cells, what will happen to cells with depleted or interfered Tpk2-Rpd3L pathway when cultured in medium with sucrose as the sole carbon source?
2. HDAC1 is a major deacetylase in mammalian cells. This study found that ectopic expression of Flag-HDAC1-S434A reduced the expression of TCA cycle genes, does the reduction in gene transcription specific to TCA cycle genes? The authors need to thoroughly analyze the global impact of PKA depletion or inhibition to the genomic recruitment of HDAC1 as well as the change of gene expression in HepG2 cells.
3. Ectopic expression of Flag-HDAC1-S434A mildly repressed HepG2 cell growth, it is an overclaim that this pathway "provides a potential target for future cancer treatment".
4. PKA is activated upon fasting in liver tissue, will fasting also induce HDAC1 phosphorylation? Studies in HepG2 cells cannot reflect the physiological involvement of this PKA-HDAC1 pathway in regulating metabolic adaptations in mammalian systems.

Reviewer #3

(Remarks to the Author)
In this paper, Dai, Zheng et al. explore the mechanisms of cellular adaptation to a change of carbon source, using mainly S.

cerevisiae as a model organism. They focus on the role of the SAGA complex and its regulation by PKA and the deacetylase Rpd3L. Using a combination of biochemistry, genomics, proteomics and metabolomics, the authors present a convincing model in which:
1. Rpd3L, but not the other Rpd3 complexes, deacetylates the SAGA subunit Ada3 to regulate genes involved in metabolism adaptation.
2. Tpk2 (PKA) phosphorylates the Rpd3 complex on Rpd3 and Ash1 and therefore inhibits the deacetylase activity of Rpd3L and the interaction of SAGA and Rpd3L.
3. Increased acetylation of SAGA in sucrose grown cells, leads to increased Histone acetylation, particularly at genes involved in metabolism. This transcriptional activation leads to a switch from glycolysis (glucose) to the TCA cycle (sucrose).
4. Similar regulation seem to occur in mammalian cells.

The work is supported by a very large and impressive amount of data on acetylation dynamics, in vivo co-IPs combined with in vitro reconstitution of interactions, identification of phosphosites and analysis of both phospho-ablating and phospho-mimicking mutations. The work is clearly interesting and well adapted to the molecular mechanism studied. Regarding the text, it is clear yet a bit dry given the length and the amount of data. It could be slightly shorten to ease its reading.

Major comments:
1. Most of the regulation proposed by the authors is through a control of the de-acetylation activity of Rpd3L and through Rdp3 interaction strength with SAGA. However, I am a bit confused by the localisations presented by the authors using antibodies. It would appear that the Rpd3L complex is mostly cytoplasmic, while SAGA is known to be mostly nuclear. The authors did not test the possibility that phosphorylation could in fact control the localisation of Rpd3L. A simple co-localisation analysis of Rpd3 and SAGA using live cells expressing fluorescently labelled proteins would be very informative to support the conclusions of the authors. This is particularly true for the S-to-D mutants of Rpd3.
2. Fig. 2F: the de-acetylation curves of the WT appear quite different from all the other WT curves presented in the work. This is slightly odd as the tpk2Δ data would be very similar to the curve of Rpd3L presented in Fig1C.
3. While the mammalian work may argue that the regulation of metabolism presented in yeast seems to be conserved, I am not certain it really adds to the study. The yeast work is already huge and more convincing in that the authors used a switch in carbon source as a biological paradigm. In the mammalian work, the phenotypic consequence of mutating HDAC1 (S to A) is that the cells grow poorly in general. As such, my feeling is that the study would benefit from removing these data such that they could be consolidated and used for another publication.

Minor comments:
1. S. cerevisiae should be mentionned in the abstract.
2. In the introduction, it reads weird that the AMPK, mTOR and PKA pathways are described only from a mammalian perspective, while the work is mostly performed on yeast.
3. Fig 6f: the I from IP is missing.
4. the Ash1-2SA mutant is more effective at deacetylating Ada3, and the 2SD does not deacetylate. However, in both cases, there is an increase in de-acetylation and the Ash1-2SA is over-active in glucose. Why? It did not seem to be the case in the tpk2Δ cells.
5. Line 380 to 381. I suppose the authors meant "when cells were shifted from glucose to sucrose". The YP to YP glucose or YP sucrose relate to Fig S7b.
6. Because the double Rpd3-2SA Ash1-2SA mutant does not show a further reduction of H3 acetylation, the authors conclude that Rdp3-2SA may play a more prominent role than Ash1-2SA (lines 384-386). However, the levels of acetylation of both single mutants are similar in fig. 7C. Therefore, I wonder why the authors reach or propose this conclusion?
7. The type of mammalian cells used in this work should be stated in the main text.
8. In Fig. 8b the blot shown appears to demonstrate that HDAC8 knockdown leads to a complete disappearance. This is not discussed at all.
9. Fig 8H Nucleosomes and not nuclesomes.

This is a review from Fabrice Caudron.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The Authors did an excellent job improving the manuscript according to the suggestions provided in the first round of evaluation. I think this is a nice piece of work, investigating the role of Rpd3 phosphorylation in yeast and of the mammalian ortholog HDAC1 in the metabolic adaptation. The results have relevant implications in the regulation of metabolic pathways in mammals, as nicely shown with the experiments performed in HepG2 cells.
As far as I am concerned, I have only a couple of doubts, and I would like to ask the Authors to make a final effort to address and improve the manuscript.

1. In Figure 7h and 7i they measured the mRNA levels and histone acetylation of some genes supposedly involved in TCA cycle in yeast. However, among these genes they tested IDP2, which encodes the cytosolic NADP-specific isocitrate dehydrogenase and not the mitochondrial isocitrate dehydrogenase, the one actually involved in TCA cycle. Can you, please, explain?

2. As suggested after the first round of evaluation, the Authors tried to detect the nuclear form of ACLY using immunofluorescence microscopy. They reported that 8-Bromo-cAMP treatment “slightly increased nuclear localization of ACLY in both Flag-HDAC1 and Flag-HDAC1-S434A HepG2 cells (Supplementary Fig. 12a)”, however they did not quantify the change. According to the images in Supplementary Fig. 12a, although there seems to be a slight increase, the difference may not be significant as the difference is not striking. An alternative to prove the importance of providing the substrate acetyl-CoA for histone acetylation could be to measure the expression of ACLY and of the mitochondrial citrate transporter SLC25A1. This could be done quickly by qPCR with RNA the Authors have already used to quantify the levels of other mRNAs reported in supplementary Figure 10b and 10c.

Reviewer #2

(Remarks to the Author)
The authors have nicely addressed my previous concerns.

Reviewer #3

(Remarks to the Author)
The authors have answered all my questions.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The Authors addressed my comments. I have no further requests

Point-by-point response to the referees' comments

REVIEWER COMMENTS
Our responses are in blue.

Reviewer #1 (Remarks to the Author):

The manuscript authored by Wenjing Dai et al. deals with the phosphorylation of yeast Rpd3L consequent to shift to nutritional status in the presence of sucrose as energy source. The Authors aimed to unveil how yeast cells sense extracellular sucrose signals and respond with alterations of histone modifying enzymes. They identified the GPCR Gpr1 as the nutrient sensor of sucrose with high affinity binding for this disaccharide in yeast. Furthermore, they described a signaling pathway that directly connect nutrient sensing with chromatin modifications. In detail, they found that Tpk2-catalyzed Rpd3 phosphorylation impairs the deacetylase activity of Rpd3 and diminished Ada3 deacetylation within SAGA. This effect is mediated by the direct interaction between Rpd3L specific subunit Ash1 and SAGA subunit Ubp8, thus connecting extracellular sucrose with repression of Rpd3L and auto-acetylation of SAGA. In the final series of experiments the Authors found that PKA phosphorylates the class I histone deacetylase (HDAC) HDAC1 in mammalian cells, and by reducing its deacetylase activity it increases histone acetylation and enhances TCA cycle. Along this line, the Authors hypothesized that PKA could promote tumorigenesis by phosphorylating and inhibiting HDAC1, since it has been reported that PKA promotes hepatocellular carcinoma invasion and metastasis by phosphorylating CIP4.
The Authors performed a very detailed analysis of the biochemical mechanisms of Rpd3/HDAC1 regulation by nutrients, which allowed to identify a signaling pathway linking extracellular nutrients to epigenome modifications and metabolic regulation.

We would like to thank this reviewer for constructive comments and suggestions. In the revised manuscript, we have addressed all the comments raised by the reviewer and revised the manuscript accordingly. We really appreciate the reviewer's comments on our statistical analysis. We agree with this suggestion and re-analyzed our data using two-way ANNOVA as suggested. The point-by-point answers to all the comments raised by the reviewer are enumerated below.

I have the following comments to the manuscript:

1. In many figure panels the Authors used two-tailed t-tests which does not seem appropriate. For instance, in supplementary figure 2d the Authors report "two-tailed t-tests were used for statistical analysis" in the legend. However, in this case there are more than two groups in the experiment, therefore a two-way ANOVA would seem more appropriate as the variables in this experiment are the treatment with YP,

YP+glucose, YP+sucrose and the genotype (wt, pde1mutant, pde2mutant). Statistical analysis should be reviewed for data reported in this graph. Also, the comparisons should be clearly stated in the figure legend (e.g., **, $p<0.01$; ***, $p<0.001$ refer to what comparisons?). Please, review the statistical analyses in all figures and apply the appropriate test accordingly.

We agree with the reviewer that a two-way ANOVA is more appropriate for statistical analysis of some data in the figures. As per requested, we reviewed the statistical analysis and performed a two-way ANOVA analysis for data in Fig. 1b, 1c, 1e, 2e, 2f, 2i, 2j, 3i, 4a-4f, 5g, 5i, 6f, 6h, 6i, 7c, 7d, 7e, 7h, 7i, 7k, 8h, 8k, 8m, 8n; Supplementary Fig. 1d, 1e, 2a-2d, 2h-2j, 4c, 4e-4h, 5c, 5j, 6d, 7b-7e, 7g-7h, 10b-10c, 10e, 10g, 11, 12b. We also clearly labelled the comparisons in the figure legends.

2. In figure 2i the Authors show that loss of Gpr1 abrogated sucrose-induced increase of cAMP. What happens to cAMP levels if Gpa2 is mutated? In the present version of the manuscript the Authors only show that loss of Gpa2 attenuated sucrose-induced Ada3-K14ac and Ada3-K182ac. The Authors should also show whether loss of Gpa2 blunts the effect of sucrose on cAMP levels.

We thank the reviewer for this point. We examined the effect of sucrose on cAMP levels in WT and *gpa2Δ* mutant. The results showed that loss of Gpa2 abrogated the inducing effect of sucrose on cAMP levels, which is similar to *gpr1Δ* mutant (Fig. 2i).

Line 205: Changed "Loss of Gpr1 abrogated sucrose-induced increase of cAMP (Fig. 2i)." to "Loss of Gpr1 and Gpa2 abrogated sucrose-induced increase of cAMP (Fig. 2i)."

3. For the sake of clarity: it seems that Rpd3-2SA be a double mutant on both S50 and S354 amino acid residues. This should be more clearly explained. I could not find any details about this mutant in the "Methods" section. Likewise, for Ash1-2SA mutant. In general, there is no description of how these mutants have been made in the "Methods" section.

We thank the reviewer for this point. In the revised manuscript, we described Rpd3-2SA as a double mutant on both S50 and S354 of Rpd3 in the results section. In addition, we described how we constructed this mutant in the method section. We also clearly described Ash1-2SA mutant in both results and method sections.

Lines 228-230: Changed "Immunoblot and immunofluorescence analysis with these two antibodies detected phosphorylated S50 and S354 in WT Rpd3 but not in Rpd3-S50A S354A" to "Immunoblot and immunofluorescence analysis with these two antibodies detected phosphorylated S50 and S354 in WT Rpd3 but not in Rpd3 double mutant on both S50 and S354 residues (Rpd3-S50A S354A, which we refer to hereafter

as Rpd3-2SA)".

Lines 342-344: Changed "We thus mutated these two residues to construct Ash1-S149A, Ash1-S388A, Ash1-S149A S388A (Ash1-2SA) mutants." to "We thus mutated these two residues to construct Ash1-S149A, Ash1-S388A, Ash1-S149A S388A (Ash1 double mutant on both S149 and S388 residues, which we refer to hereafter as Ash1-2SA) mutants."

Lines 650-662: Added "The endogenously expressed Rpd3-2SA (Rpd3-S50A S354A) mutant was constructed by homologous recombination of PCR fragments as follows: The *Saccharomyces cerevisiae* Rpd3 was cloned and inserted into plasmid pRS316 to generate pRS316-Rpd3. The pRS316-Rpd3-S50A S354A plasmid was obtained by site-directed mutagenesis and used as the template to amplify the Rpd3-S50A S354A fragment with a selectable marker *URA3* and 60 nt homologous sequence to endogenous *RPD3* flanking sequence. The PCR fragment was then integrated into a *RPD3* knockout strain through homologous recombination. Successful transformants were screened to obtain the Rpd3-S50A S354A mutant. The endogenously expressed Ash1-2SA (Ash1-S149A S388A) mutant was constructed using the similar approach except that the Ash1-S149A S388A fragment with a selectable marker *HIS3* and 60 nt homologous sequence to endogenous *ASH1* flanking sequence was amplified by PCR and then integrated into a *ASH1* knockout strain through homologous recombination."

4. Figure 3f: these images must be quantified. It is not clear from the immunofluorescence images whether sucrose increased phosphorylation of Rpd3 on S50 and S354 in wt cells when compared to cells treated with glucose.

In the revised manuscript, we quantified the images in Fig. 3f. It revealed that sucrose treatment significantly increased phosphorylation of Rpd3 on S50 and S354 in WT cells when compared to glucose treatment (Fig. 3f).

5. Figure 4b reporting immunofluorescence analysis of Ada3 acetylation: the images must be quantified.

In the revised manuscript, we quantified the images in Fig. 4b. It revealed that sucrose induced Ada3-K14ac and Ada3-K182ac in WT but not in Rpd3-2SA and *tpk2Δ* mutants (Fig. 4b).

6. Again, I have reservation about the statistical analyses in the figures 4a and 4c: the Authors performed two-tailed t-tests. However, the variables in these two graphs are two (i.e., treatment glucose vs. sucrose, and wt vs. mutant), therefore a two-way ANOVA should be applied. In addition, it is not clear to what comparisons the

statistically significant differences refer according to the figure legend.

We agree with the reviewer for this point. In the revised manuscript, we used a two-way ANOVA for statistical analysis of Fig. 4a and 4c as requested. We also clearly indicated what comparisons the statistically significant differences refer in the figures.

7. The western blot in supplementary figure 4b, showing that the mutation of Rpd3-2SD rescued the reduced Ada3-K14ac and Ada3-K182ac in tpk2Δ mutant, should be quantified.

In the revised manuscript, we performed additional repeats for the western blots in supplementary Fig. 4b and quantified the data. We also performed a two-way ANOVA analysis (Supplementary Fig. 4c).

8. In experiments reporting kinetic analysis of protein phosphorylation or acetylation (e.g. figure 1b, 1c, 1e, 2f, 3i, 4d, 4e, 4f etc. and in supplementary figures as well) the Authors added P values in the graphs, but I could not find what these P values refer to. Is it by perhaps referred to AUCs? Please, clearly specify them in all figure legends.

We feel sorry for not indicating clearly what the *P* values refer to. For kinetic analysis graphs mentioned, the *P* values refer to the statistical difference of Ada3-K14ac/Ada3 and Ada3-K182ac/Ada3 between two groups. We analyzed the data by a two-way ANOVA analysis and indicated what these *P* values refer to in figure legends.

Lines 1079-1080: Added "*P* values refer to the statistical difference of Ada3-K14ac/Ada3 and Ada3-K182ac/Ada3 between Wt Rpd3 and Rpd3 *ash1Δ* mutant."

Lines 1107-1109: Added "*P* values refer to the statistical difference of Ada3-K14ac/Ada3 and Ada3-K182ac/Ada3 between Wt Rpd3L and Rpd3L *tpk2Δ* mutant."

9. The western blots in supplementary figure 4e and 4f should be quantified.

In the revised manuscript, we performed additional repeats for kinetic assays in Supplementary Fig. 4e and 4f and quantified the data. We also performed a two-way ANOVA analysis of the quantified data (Now in Supplementary Fig. 4g and 4h).

10. There are several western blots of co-IP experiments that should be quantified. The Authors make statements about enhanced or reduced interactions depending on mutant or culture conditions. For instance, in figure 6f they claim that "In vivo Co-IP showed that mutation of Ash1-2SA enhanced the intracellular interaction with Ubp8 and Ada3

within SAGA and mutation of Ash1-2SD reduced its interaction with Ubp8 and Ada3 within SAGA". These differences are often subtle and hard to appreciate; quantification should be performed to consolidate the statements, which otherwise would be somewhat questionable. Another example is in figure 7a and supplementary figure 7a, where the Authors state that loss of Tpk2 reduced Ada3 dimerization. Please, note that there are other similar cases in the manuscript.

To address this reviewer's comments, we made the following changes:

(1) We performed additional repeats for the Co-IP in Fig. 6f and quantified the data. We also performed a two-way ANOVA analysis of the quantified data (Fig. 6f). The data showed that mutation of Ash1-2SA enhanced the intracellular interaction with Ubp8 and Ada3 within SAGA and mutation of Ash1-2SD reduced its interaction with Ubp8 and Ada3 within SAGA.

(2) We performed additional repeats for the Co-IP in Fig. 7a and Supplementary Fig. 7a, and quantified the data as requested. We also performed a two tailed unpaired *t*-test analysis of the quantified data (Fig. 7a and Supplementary Fig. 7a). The data showed that mutation of Rpd3-2SA or loss of Tpk2 reduced Ada3 dimerization.

(3) In addition, we performed additional repeats for the Co-IP in Fig. 2d, 2g, 5g, 6b, 6f, 6g, 6h, 7b; Supplementary Fig. 5c, 6b, 7a and quantified the data. We also performed statistical analysis of the quantified data.

11. Pg 17, lines 380-381: it seems that the Authors indicated the wrong figure in the text. It should be supplementary figure 7c and not figure 7c, when presenting the data relative to global levels of H3K9ac and H3K14ac, which gradually increased when cells were shifted from YP medium to YP + 2% glucose medium or to YP + 2% sucrose medium. In fact, in figure 7c there is no switch from YP medium to YP medium + glucose and YP medium + sucrose. Again, here the quantification of the western blot images in supplementary figure 7c should be added.

We feel sorry for this error. It is not Figure 7c. Both Supplementary Fig. 7b and Supplementary Fig. 7c showed increased levels of H3K9ac and H3K14ac in cells when shifted from YP medium to YP + 2% glucose or to YP + 2% sucrose. We thus changed it to Supplementary Fig. 7b as it occurred first. Moreover, we performed additional repeats for the Western blots in Supplementary Fig. 7c and quantified the data as requested. We also performed a two-way ANOVA analysis of the quantified data (Supplementary Fig. 7c).

Line 386: Changed "Fig. 7c" to "Supplementary Fig. 7b".

12. In figure 7d and in supplementary figure 7d the Authors show that the sucrose-dependent-induction of SUC2 is reduced with Ada3-3KR, Rpd3-2SA, TpkaΔ and Gpr1Δ mutants. However, although the induction is reduced with mutants, sucrose can

still induce SUC2 transcripts quite significantly. Why? Is there another mechanism underlying the sucrose-mediated induction of SUC2? Also, the statistical analysis in supplementary figure 7b and supplementary figure 7d was performed with two-tailed t-tests, however, since there are more variables, two-way ANOVA should be applied. Likewise in figure 7c and in figure 7d.

We thank the reviewer for this point. Although sucrose-induced *SUC2* transcription was reduced in Ada3-3KR, Rpd3-2SA, *tpk2Δ*, and *gpr1Δ* mutants, sucrose can still induce *SUC2* transcription in these mutants (Fig. 7d; Supplementary Fig. 7d). The reason is that *SUC2* gene transcription is not only controlled by histone acetylation but also by other transcription regulators. The prominent example is the repressor complex Ssn6-Tup1, which is recruited to *SUC2* promoter to repress *SUC2* transcription via its interaction with Mig1 when cells are grown in high glucose medium (PMID: 8943325; PMID: 8008070; PMID: 7724528). When cells are grown in low glucose, Mig1 is phosphorylated and inactivated by Snf1/Snf4 serine-threonine protein kinase complex, which then derepresses *SUC2* (PMID: 7724528). These pathways work together to regulate *SUC2* transcription in response to extracellular carbon source changes. In the revised manuscript, we added these sentences in the discussion section.

As requested by the reviewer, we performed two-way ANOVA analysis for Fig. 7c, 7d, Supplementary Fig. 7b and 7d.

Lines 591-599: Added "Although sucrose-induced *SUC2* transcription was reduced in Ada3-3KR, Rpd3-2SA, *tpk2Δ*, and *gpr1Δ* mutants, sucrose can still induce *SUC2* transcription in these mutants (Fig. 7d; supplementary Fig. 7d). This may be caused by the fact that *SUC2* transcription is not only controlled by histone acetylation but also regulated by other proteins. For example, when cells are grown in high glucose medium, the Ssn6-Tup1 complex is recruited to *SUC2* promoter to repress *SUC2* transcription via its interaction with Mig1 [50-52]. When cells are grown in low glucose, Mig1 is phosphorylated and inactivated by Snf1/Snf4 serine-threonine protein kinase complex, which then derepresses *SUC2* [52]. These pathways work together to regulate *SUC2* transcription in response to extracellular carbon source changes."

13. PKA-induced phosphorylation of Rpd3/HDAC1 represses Rpd3/HDAC1 activity and leads to increased mRNA levels of genes encoding enzymes of TCA cycle in yeast and in HepG2 cells. However, the Authors only show hyperacetylation of nucleosomes on SUC2 promoter in yeast while they do not show whether the hyperacetylation occurs to the promoter/enhancer of genes of the TCA cycle in yeast treated with sucrose and in HepG2 cells treated with cAMP. This is a significant piece of information that is missing in the manuscript. It would be important to show that promoter/enhancer regions are hyperacetylated at least for some genes involved in TCA cycle in yeast and in HepG2 cells.

We thank the reviewer for this point. To address the reviewer's questions, we performed the following experiments:

(1) We performed ChIP-qPCR for histone H3K9 acetylation at the promoters of TCA cycle genes in yeast. The enrichment of histone acetylation at these gene promoters was significantly induced by sucrose treatment and mutation of Rpd3-2SA and Ash1-2SA reduced histone acetylation at these gene promoters (Fig. 7i; Supplementary Fig. 7h). Moreover, sucrose had no effect on histone acetylation at these gene promoters in Rpd3-2SA and Ash1-2SA mutants (Fig. 7i; Supplementary Fig. 7h).

(2) We examined H3K9ac at the promoters of TCA cycle genes when HepG2 cells were treated with cAMP analog 8-Bromo-cAMP. It revealed that H3K9ac was increased at these gene promoters by 8-Bromo-cAMP treatment in Flag-HDAC1 (Supplementary Fig. 10e). Mutation of HDAC1-S434A reduced H3K9ac at these gene promoters (Supplementary Fig. 10e). In addition, 8-Bromo-cAMP treatment had no effect on H3K9ac in Flag-HDAC1-S434A mutant (Supplementary Fig. 10e).

Lines 425-427: Added "Sucrose had no effect on histone acetylation at these gene promoters in Rpd3-2SA and Ash1-2SA mutants (Fig. 7i; Supplementary Fig. 7h)."

Lines 512-515: Added "H3K9ac was increased at these gene promoters by 8-Bromo-cAMP treatment in Flag-HDAC1 (Supplementary Fig. 10e). Mutation of HDAC1-S434A reduced H3K9ac at these gene promoters and abolished the inducing effect of 8-Bromo-cAMP (Supplementary Fig. 10e)."

14. In line with the previous comment, hyperacetylation of promoter/enhancer regions requires acetyl-CoA as the acetyl donor for histone acetylation of target genes. ATP-citrate lyase (ACLY) is the enzyme providing acetyl-CoA for histone acetylation and has been demonstrated to localize also in the nucleus, where it cleaves citrate to acetyl-CoA and oxaloacetate in mammalian cells (SCIENCE, 22 May 2009, Vol 324, Issue 5930, pp. 1076-1080 DOI: 10.1126/science.1164097). The Authors should test whether treatment with PKA activator increases the amount of ACLY in nuclei of HepG2 cells. This could be easily assessed by immunofluorescence microscopy.

We thank the reviewer for this point. To address the reviewer's questions, we performed the following experiments:

(1) We analyzed the effect of PKA activator 8-Bromo-cAMP on ACLY nuclear localization by immunofluorescence. It showed that 8-Bromo-cAMP treatment slightly increased the nuclear localization of ACLY in both Flag-HDAC1 and Flag-HDAC1-S434A HepG2 cells (Supplementary Fig. 12a). No difference was observed for ACLY localization in Flag-HDAC1 and Flag-HDAC1-S434A mutant when treated with 8-Bromo-cAMP (Supplementary Fig. 12a).

(2) We examined the effect of 8-Bromo-cAMP on histone acetylation in Flag-HDAC1 and Flag-HDAC1-S434A HepG2 cells. 8-Bromo-cAMP treatment increased

histone acetylation in Flag-HDAC1 cells and mutation of HDAC1-S434A significantly reduced 8-Bromo-cAMP-induced increase of histone acetylation (Supplementary Fig. 12b), suggesting that PKA induced histone acetylation primarily by repressing HDAC1 activity.

Together, the above data suggest that although PKA activator slightly increased ACLY nuclear localization, PKA induced histone acetylation primarily by repressing HDAC1 activity. We added these sentences in the discussion section in the revised manuscript.

Lines 626-634: Added "ATP-citrate lyase (ACLY) is the enzyme providing acetyl-CoA for histone acetylation and has been demonstrated to localize in the nucleus in mammalian cells [55]. We also examined the effect of treatment with PKA activator 8-Bromo-cAMP on ACLY localization in nuclei of HepG2 cells. It revealed that 8-Bromo-cAMP treatment slightly increased the nuclear localization of ACLY in both HDAC1-Flag and HDAC1-S434A-Flag mutant (Supplementary Fig. 12a). 8-Bromo-cAMP treatment increased histone acetylation in Flag-HDAC1 cells and mutation of HDAC1-S434A significantly reduced 8-Bromo-cAMP-induced increase of histone acetylation (Supplementary Fig. 12b), suggesting that PKA induced histone acetylation primarily by repressing HDAC1 activity."

Minor points:

1. Supplementary figure 7i: it is not clear what "1N" and "2N" stand for. Please, specify in the figure legend to help the reader.

We thank the reviewer for this point. We added the description of what "1N" and "2N" stand for in the figure legends.

2. The abbreviations of genes tested in figure 7 and 8 (and the corresponding supplementary figures) should be spelled out.

We thank the reviewer for this point. For those abbreviated genes, we spelled their full name in the figure legends of Fig. 7, Fig. 8, Supplementary Fig. 7, Supplementary Fig. 10.

3. In supplementary figure 8g HepG2 cells were treated with cAMP. What is the chemical compound the Authors used? Is it a stable analog (e.g., 8-Br-cAMP)? Please, specify it in the methods section as well as in the figure legend.

We thank the reviewer for this point. We used cAMP analog 8-Bromo-cAMP. We specify it in both results and method section.

Lines 507-510: Changed “In contrast, treatment with cAMP dramatically increased the expression of these TCA cycle genes in Flag-HDAC1 cells and marginally induced TCA cycle gene expression in Flag-HDAC1-S434A cells (Supplementary Fig. 8g).” to “In contrast, treatment with 8-Bromo-cAMP dramatically increased the expression of these TCA cycle genes in Flag-HDAC1 cells and marginally induced TCA cycle gene expression in Flag-HDAC1-S434A cells (Supplementary Fig. 10c).”

Line 669: Added “For cAMP treatment, the stable analog 8-Bromo-cAMP was used.”

Lines 825-826: Added “For ChIP assays in HepG2 cells, Flag-HDAC1 and Flag-HDAC1-S434A HepG2 cells were treated with or without 10 μM 8-Bromo-cAMP (HY-12306A, MCE) for 24 h.”

Lines 840-842: Added “Flag-HDAC1 and Flag-HDAC1-S434A HepG2 cells treated with 10 μM H89 (HY-15979, MCE) or 10 μM 8-Bromo-cAMP (HY-12306A, MCE) for 24 h.”

Reviewer #2 (Remarks to the Author):

In this study, Dai et al discovered that PKA can phosphorylate Rpd3/HDAC1 and regulate the expression of metabolic genes, which is crucial in mediating the metabolic adaptation of yeast, especially when cells are cultured in medium with sucrose as the sole carbon source. In general, the findings are interesting, and the data are solid. I have some comments regarding the functional contribution of this pathway to the cell fate change and its conservation in mammals.

We thank the reviewer for recognition of our work. In the revised manuscript, we performed additional experiments to address the functional contribution of PKA-catalyzed Rpd3/HDAC1 to cell fate. In addition, we studied the physiological involvement of this PKA-HDAC1 pathway in regulating metabolic adaptations in mice liver tissue upon fasting condition. The point-by-point answers to all the issues raised by the reviewer are enumerated below.

1. What is the functional contribution of this Tpk2-Rpd3L pathway to the yeast cell fate change? Since this pathway is considered important for the metabolic adaptation of cells, what will happen to cells with depleted or interfered Tpk2-Rpd3L pathway when cultured in medium with sucrose as the sole carbon source?

We thank the reviewer for this point. To explore the effect of blocking Tpk2-Rpd3L pathway on cell fate when cultured in medium with sucrose as the sole carbon source, we performed the following experiments:

(1) We examined the growth of WT, Rpd3-2SA, and Ash1-2SA mutants when grown in YP + 2% sucrose medium. Our data showed that compared with WT, Rpd3-2SA, and Ash1-2SA mutants grew slowly (Supplementary Fig. 8b).
(2) We examined the tolerance of WT, Rpd3-2SA and Ash1-2SA to oxidative stress and salt stress by treating cells with 0.1% H_2O_2 and 5 M NaCl, respectively. Both Rpd3-2SA and Ash1-2SA mutants displayed reduced survival after H_2O_2 and NaCl treatment (Supplementary Fig. 8c, d).
(3) We examined the effect of these mutations on cell apoptosis. Chronological aging can be used to study the physiological scenario of apoptosis induction in yeast (*PLoS Genet* 10, e1004095, 2014) and we thus studied the effect of WT, Rpd3-2SA and Ash1-2SA on the chronological aging of yeast cells. Rpd3-2SA and Ash1-2SA mutant cells showed an early onset of cell death during chronological aging when compared to WT cells (Supplementary Fig. 8e). Apoptosis is frequently accompanied by an accumulation of reactive oxygen species (ROS), which is an early step in the apoptotic process. Dihydroethidium (DHE) staining of Rpd3-2SA and Ash1-2SA mutant cells grown in YP + 2% sucrose medium for 3 days showed increased accumulation of ROS (Supplementary Fig. 8f). TUNEL staining revealed that Rpd3-2SA and Ash1-2SA cells showed increased DNA fragmentation (Supplementary Fig. 8f).

Based on the above results, we concluded that Tpk2-mediated Rpd3L phosphorylation improves cell survival under environmental stress conditions.

Lines 436-438: Added “Rpd3-2SA and Ash1-2SA mutants also displayed slower cell growth in sucrose-containing medium (Supplementary Fig. 8b).”

Lines 441-449: Added “We then examined the effect of these mutations on cell apoptosis. Chronological aging can be used to study the physiological scenario of apoptosis induction in yeast [38,39]. Compared to WT cells, Rpd3-2SA and Ash1-2SA mutants showed an early onset of cell death during chronological aging when grown in sucrose-containing medium (Supplementary Fig. 8e). Dihydroethidium (DHE) staining of Rpd3-2SA and Ash1-2SA mutants grown in YP + 2% sucrose medium for 3 days showed an increased accumulation of reactive oxygen species (ROS) (Supplementary Fig. 8f), which is an early step in the apoptotic process. TUNEL staining revealed that Rpd3-2SA and Ash1-2SA mutants had increased DNA fragmentation (Supplementary Fig. 8f).”

2. HDAC1 is a major deacetylase in mammalian cells. This study found that ectopic expression of Flag-HDAC1-S434A reduced the expression of TCA cycle genes, does the reduction in gene transcription specific to TCA cycle genes? The authors need to thoroughly analyze the global impact of PKA depletion or inhibition to the genomic recruitment of HDAC1 as well as the change of gene expression in HepG2 cells.

We thank the reviewer for this point. To examine the global effect of the PKA-HDAC1

pathway on gene transcription, we performed the following experiments:

(1) We performed RNA-seq analysis of Flag-HDAC1 and Flag-HDAC1-S434A HepG2 cells treated with or without H89. H89 treatment reduced the transcription of 3106 genes, among which 2146 genes were repressed in Flag-HDAC1-S434A mutant (Supplementary Fig. 9f, g). As expected, the transcription of genes involved in TCA cycle and oxidative phosphorylation was reduced by H89 and Flag-HDAC1-S434A. H89 had no significant effect on transcription of these genes in Flag-HDAC1-S434A mutant (Supplementary Fig. 10a), suggesting that blocking PKA-catalyzed HDAC1-S434 phosphorylation repressed these gene expression and this repression is not only restricted to TCA cycle genes.

(2) We performed Cleavage Under Targets & Tagmentation (CUT & Tag) for HDAC1 when HepG2 cells were treated with or without H89. It revealed that HDAC1 directly bound 6837 genes, among which 475 genes were down-regulated by HDAC1-S434A and H89 treatment and 680 genes were up-regulated by HDAC1-S434A and H89 treatment (Supplementary Fig. 9i). Moreover, KEGG analysis of 680 genes revealed that these genes were enriched in ribosome, pathway of neurodegeneration, Huntington disease, Amyotrophic lateral sclerosis, Alzheimer disease, Parkinson disease, prion disease, thermogenesis, oxidative phosphorylation, etc. (Supplementary Fig. 9j). KEGG analysis of 475 genes revealed that these genes were enriched in focal adhesion, parathyroid hormone synthesis, secretion and action, spinocerebellar ataxia, insulin resistance, acute myeloid leukemia (Supplementary Fig. 9j).

Together, these data confirm that the PKA-HDAC1 pathway represses the transcription of TCA cycle genes. Our data also suggest that the PKA-HDAC1 pathway regulates the transcription of genes not restricted to TCA cycle.

Lines 489-503: Added “We also performed RNA-seq analysis of Flag-HDAC1 and Flag-HDAC1-S434A HepG2 cells treated with or without H89. H89 treatment reduced the transcription of 3106 genes, among which 2146 genes were repressed in Flag-HDAC1-S434A mutant (Supplementary Fig. 9f, g). We also performed Cleavage Under Targets & Tagmentation (CUT & Tag) for HDAC1 when HepG2 cells were treated with or without H89. H89 only slightly increased the occupancy of HDAC1 at chromatin (Supplementary Fig. 9h), suggesting that PKA-catalyzed HDAC1 phosphorylation has little to no effect on its binding to chromatin. HDAC1 directly bound 6837 genes, among which 475 genes were down-regulated by HDAC1-S434A and H89 treatment and 680 genes were up-regulated by HDAC1-S434A and H89 treatment (Supplementary Fig. 9i). KEGG analysis of 680 genes revealed that these genes were enriched in ribosome, pathway of neurodegeneration, Huntington disease, Amyotrophic lateral sclerosis, Alzheimer disease, Parkinson disease, prion disease, thermogenesis, oxidative phosphorylation, etc. (Supplementary Fig. 9j). KEGG analysis of 475 genes revealed that these genes were enriched in focal adhesion, parathyroid hormone synthesis, secretion and action, spinocerebellar ataxia, insulin resistance, acute myeloid leukemia (Supplementary Fig. 9j).”

3. Ectopic expression of Flag-HDAC1-S434A mildly repressed HepG2 cell growth, it is an overclaim that this pathway "provides a potential target for future cancer treatment".

We thank the reviewer for this point. We removed "PKA-catalyzed HDAC1 phosphorylation may present a possible target to manipulate HDAC1 activity in developing potential cancer treatment strategies" in the abstract section and discussion section.

Lines 40-41: Changed "Our work hence reveals a conserved role of PKA in regulating Rpd3/HDAC1 and metabolism adaptation and provides a potential target for future cancer treatment." to "Our work hence reveals a conserved role of PKA in regulating Rpd3/HDAC1 and metabolism adaptation."

Line 645: Deleted "PKA-catalyzed HDAC1 phosphorylation may present a possible target to manipulate HDAC1 activity in developing potential cancer treatment strategies."

4. PKA is activated upon fasting in liver tissue, will fasting also induce HDAC1 phosphorylation? Studies in HepG2 cells cannot reflect the physiological involvement of this PKA-HDAC1 pathway in regulating metabolic adaptations in mammalian systems.

We thank the reviewer for this point. To investigate the physiological significance of PKA-HDAC1 pathway in regulating metabolic adaptations in mammalian system, we performed the following experiments:

(1) We examined whether PKA phosphorylates HDAC1 in liver tissues when mice were fasted. Mice were fed *ad libitum* or fasted for 12 h (5 mice per group). We then immunoprecipitated HDAC1 from liver extracts and examined HDAC1 phosphorylation. It revealed that fasting can induce HDAC1 phosphorylation (Fig. 8m). To further confirm it is PKA activity, we also injected fed or fasted mice with PKA inhibitor H89. It revealed that H89 reduced fasting-induced HDAC1 phosphorylation (Fig. 8m).
(2) We examined the effect of PKA-HDAC1 pathway on TCA cycle genes expression. We extracted RNA from the liver tissues of fed or fasting mice treated with or without H89 and performed qRT-PCR. It revealed that fasting can induce the expression of TCA cycle genes and H89 treatment repressed these gene expression (Fig. 8n).
(3) We examined the effect of PKA-HDAC1 pathway on TCA cycle metabolites. We extracted metabolites from the liver tissues of fed or fasting mice treated with or without H89. It revealed that fasting increased the intracellular amount of TCA cycle metabolites and H89 treatment reduced TCA cycle metabolites (Fig. 8o;

Supplementary Fig. 11).
Together, these data suggest that under fasting condition, PKA is activated to phosphorylate HDAC1, which derepresses the inhibitory effect of HDAC1 on TCA cycle gene expression and enhances metabolic adaptations.

Lines 519-526: Added "We then investigated the physiological significance of PKA-HDAC1 pathway in regulating TCA cycle in mammalian system. C57BL/6J mice were fasted for 12 h, which has been reported to induce PKA [43]. HDAC1 was immunoprecipitated from the liver tissues and immunoblots revealed that HDAC1 phosphorylation was induced after fasting and this phosphorylation was abolished upon treatment with PKA inhibitor H89 (Fig. 8m). Consistently, the expression of TCA cycle genes as well as the intracellular amount of TCA cycle metabolites were increased under fasting condition and this inducing effect was abolished by H89 treatment (Fig. 8n and 8o; Supplementary Fig. 11)."

Reviewer #3 (Remarks to the Author):

In this paper, Dai, Zheng et al. explore the mechanisms of cellular adaptation to a change of carbon source, using mainly S. cerevisiae as a model organism. They focus on the role of the SAGA complex and its regulation by PKA and the deacetylase Rpd3L. Using a combination of biochemistry, genomics, proteomics and metabolomics, the authors present a convincing model in which:

1. Rpd3L, but not the other Rpd3 complexes, deacetylates the SAGA subunit Ada3 to regulate genes involved in metabolism adaptation.

2. Tpk2 (PKA) phosphorylates the Rpd3 complex on Rpd3 and Ash1 and therefore inhibits the deacetylase activity of Rpd3L and the interaction of SAGA and Rpd3L.

3. Increased acetylation of SAGA in sucrose grown cells, leads to increased Histone acetylation, particularly at genes involved in metabolism. This transcriptional activation leads to a switch from glycolysis (glucose) to the TCA cycle (sucrose).

4. Similar regulation seem to occur in mammalian cells.

The work is supported by a very large and impressive amount of data on acetylation dynamics, in vivo co-IPs combined with in vitro reconstitution of interactions, identification of phosphosites and analysis of both phospho-ablating and phospho-mimicking mutations. The work is clearly interesting and well adapted to the molecular mechanism studied. Regarding the text, it is clear yet a bit dry given the length and the amount of data. It could be slightly shorten to ease its reading.

We thank the reviewer for recognition of our work. We especially thank this reviewer for constructive suggestions. In the revised manuscript, we tried our best to shorten the manuscript. We sincerely appreciate the reviewer's suggestion to remove the mammalian work and we agree with your opinion that this part could be removed. However, as reviewer 2 requested us to perform additional mammalian cell experiments as well as animal experiments, we thus contacted with the handling editor and she asked us to keep this part. Nonetheless, we are happy to change the manuscript if you have any better suggestions. The point-by-point answers to all the issues raised by the reviewer are enumerated below.

Major comments:

1. Most of the regulation proposed by the authors is through a control of the de-acetylation activity of Rpd3L and through Rdp3 interaction strength with SAGA. However, I am a bit confused by the localisations presented by the authors using antibodies. It would appear that the Rpd3L complex is mostly cytoplasmic, while SAGA is known to be mostly nuclear. The authors did not test the possibility that phosphorylation could in fact control the localisation of Rpd3L. A simple co-localisation analysis of Rpd3 and SAGA using live cells expressing fluorescently labelled proteins would be very informative to support the conclusions of the authors. This is particularly true for the S-to-D mutants of Rpd3.

We thank the reviewer for this good point. Our immunofluorescence experiments showed that Rpd3 exists both in cytoplasm and nucleus (Fig. 3f). As Rpd3 exists in three distinct complexes (Rpd3L, Rpd3S, Rpd3μ) inside yeast cells, the localization of Rpd3 is hence not equal to the localization of Rpd3L. To address your questions, we performed the following experiments:

(1) We performed fluorescence microscopy for cells expressing Rxt2-GFP to indicate the localization of Rpd3L. We observed that Rxt2-GFP mainly localized in the nucleus (Supplementary Fig. 4d). Meanwhile, we compared the localization of Rxt2-GFP in WT, Rpd3-2SA, and Rpd3-2SD mutants when cells were grown in sucrose-containing medium. We can clearly see that Rpd3 phosphorylation does not affect the localization of Rpd3L (Supplementary Fig. 4d).

(2) We performed fluorescence microscopy for cells expressing Rxt2-GFP and Ada3-tdTomato. We observed the co-localization of Rxt2-GFP and Ada3-tdTomato and this co-localization was unaffected by mutation of Rpd3-2SA, and Rpd3-2SD when grown in sucrose-containing medium (Supplementary Fig. 5d).

Together, these data suggest that Rpd3L colocalizes with SAGA in the nucleus. Tpk2-catalyzed Rpd3 phosphorylation had no effect on the nucleus localization of Rpd3L.

Lines 259-261: Added "We then examined the effect of Tpk2-mediated Rpd3 phosphorylation on localization of Rpd3L complex by fluorescence microscopy of Rxt2-GFP cells. Mutation of Rpd3-2SA or Rpd3-2SD had no effect on nuclear localization of Rpd3L (Supplementary Fig. 4d)."

Lines 297-299: Added "Consistently, mutation of Rpd3-2SA or Rpd3-2SD had no effect on co-localization of Rpd3L (Rxt2-GFP) and Ada3-tdTomato (Ada3 tagged with tdTomato) (Supplementary Fig. 5d)".

2. Fig. 2F: the de-acetylation curves of the WT appear quite different from all the other WT curves presented in the work. This is slightly odd as the tpk2Δ data would be very similar to the curve of Rpd3L presented in Fig1C.

We thank the reviewer for this point. The deacetylation curves were obtained based on the quantification of immunoblots. Depending on the quality of immunoblots, there may be some fluctuations for WT between different batches of experiments. We agree with the reviewer that the deacetylation curves of the WT in Fig. 2f appear different with other WT curves. We hence re-performed the deacetylation assay for Fig. 2f and Fig. 1c and quantified the kinetic curves. Our data showed that the deacetylation curves of WT is similar to the deacetylation curves of Rpd3L (Fig. 1c and Fig. 2f). Rpd3L *tpk2*Δ mutant showed more rapid deacetylation curves (Fig. 2f).

3. While the mammalian work may argue that the regulation of metabolism presented in yeast seems to be conserved, I am not certain it really adds to the study. The yeast work is already huge and more convincing in that the authors used a switch in carbon source as a biological paradigm. In the mammalian work, the phenotypic consequence of mutating HDAC1 (S to A) is that the cells grow poorly in general. As such, my feeling is that the study would benefit from removing these data such that they could be consolidated and used for another publication.

We sincerely thank the reviewer for recognition of our yeast work and we agree with your opinion that the mammalian work could be removed. As reviewer 2 requested us to perform additional mammalian cell and animal experiments, we struggled whether to include the mammalian data. We thus contacted with the handling editor and she asked us to keep this part. By performing extra mammalian experiments, we think this part has been improved and can indicate our mechanism is conserved from yeast to mammals. Nonetheless, we are happy to change the manuscript if you have any better suggestions.

Minor comments:
1. S. cerevisiae should be mentioned in the abstract.

We thank the reviewer for this point. We added "*Saccharomyces cerevisiae*" in the abstract.

Lines 24-27: Changed "The histone acetyltransferase SAGA (Spt-Ada-Gcn5-acetyltransferase) complex has been reported to acetylate its subunit Ada3 and form homo-dimers to enhance its ability to acetylate nucleosomes and facilitate metabolic gene transcription." to "The yeast histone acetyltransferase SAGA (Spt-Ada-Gcn5-acetyltransferase) complex has been reported to acetylate its subunit Ada3 and form homo-dimers to enhance its ability to acetylate nucleosomes and facilitate metabolic gene transcription."

Lines 28-30: Changed "Here, we found that SAGA is deacetylated by Rpd3L complex and uncovered how its deacetylase activity is repressed by nutrient sensor protein kinase A (PKA)." to "Here, we found that SAGA is deacetylated by Rpd3L complex and uncovered how its deacetylase activity is repressed by nutrient sensor protein kinase A (PKA) in *Saccharomyces cerevisiae*."

2. In the introduction, it reads weird that the AMPK, mTOR and PKA pathways are described only from a mammalian perspective, while the work is mostly performed on yeast.

We thank the reviewer for this point. In the revised manuscript, we described AMPK, mTOR, and PKA from both yeast and mammalian perspectives.

Lines 74-76: Added "The yeast AMPK homolog is Snf1, which is activated under conditions of glucose limitation to maintain energy homeostasis and control responses to environmental stresses [13]."

Lines 78-81: Added "In *Saccharomyces cerevisiae*, TOR kinases assemble into two distinct complexes: TOR complex 1 (TORC1) and TOR complex 2 (TORC2). Only TORC1 is rapamycin sensitive and promotes anabolism and cell growth [16]."

Lines 84-86: Added "The yeast PKA holoenzyme is composed of a regulatory subunit Bcy1 dimer and two catalytic subunits encoded by *TPK1*, *TPK2* and *TPK3* [18]. Upon binding of cAMP to Bcy1, the PKA tetramer dissociates into active Tpk catalytic subunits."

3. Fig 6f: the I from IP is missing.

We thank the reviewer for this point. We made changes for Fig. 6f as requested.

4. the Ash1-2SA mutant is more effective at deacetylating Ada3, and the 2SD does not deacetylate. However, in both cases, there is an increase in de-acetylation and the Ash1-2SA is over-active in glucose. Why? It did not seem to be the case in the *tpk2*Δ cells.

We thank the reviewer for this point. For Ash1-2SA mutant, there was an increase of Ada3 deacetylation even when cells were grown in glucose-containing medium (Fig. 6i), which could be due to enhanced interaction between SAGA and Rpd3L (Fig. 6f). Similar to Ash1-2SA mutant, we also observed an increase of Ada3 deacetylation in *tpk2*Δ mutant even when cells were grown in glucose-containing medium (Fig. 2e). The difference between Ash1-2SA and *tpk2*Δ mutants is that there was no change of Ada3 deacetylation when *tpk2*Δ mutant was shifted from glucose-containing medium to sucrose-containing medium (Fig. 2e), but there was still an increase of Ada3 acetylation when Ash1-2SA mutant was shifted from glucose-containing medium to sucrose-containing medium (Fig. 6i). As for the difference between Ash1-2SA and *tpk2*Δ mutants, we think there are four possibilities:

(1) When cells were shifted from glucose to sucrose, the changes of Ada3 deacetylation are determined by a combination of Tpk2-catalyzed Rpd3 phosphorylation and Ash1 phosphorylation. Therefore, Tpk2-phosphorylation Rpd3 may contribute to the increase of Ada3 acetylation in Ash1-2SA mutant.

(2) Western blots may not be that sensitive to differentiate the subtle difference between Ash1-2SA and *tpk2*Δ mutants.

(3) Mutation of Ash1-2SA is not equal to loss of Tpk2-catalyzed Ash1 phosphorylation. Our data showed that Tpk2 phosphorylates Ash1 at serine 149 and serine 388, and mutation of Ash1-2SA disrupts Tpk2-catalyzed Ash1 phosphorylation. We cannot rule out that Ash1 S149 and S388 residues also play a role in regulating Ada3 deacetylation.

(4) Tpk2 may have other potential substrates affecting Ada3 acetylation. Although our data support that Tpk2 promotes Ada3 acetylation primarily by phosphorylating Rpd3 and Ash1, we cannot rule out the possibility that Tpk2 may regulate other proteins to indirectly affect Ada3 acetylation.

5. Line 380 to 381. I suppose the authors meant "when cells were shifted from glucose to sucrose". The YP to YP glucose or YP sucrose relate to Fig S7b.

We thank the reviewer for this point. It is Supplementary Fig. 7b instead of Fig 7c. We corrected it in the result section.

Line 384-386: Changed “The global levels of H3K9ac and H3K14ac were gradually increased when cells were shifted from YP medium to YP + 2% glucose medium or to YP + 2% sucrose medium (Fig. 7c).” to “The global levels of H3K9ac and H3K14ac were gradually increased when cells were shifted from YP medium to YP + 2% glucose (YP + Glu) medium or to YP + 2% sucrose (YP + Suc) medium (Supplementary Fig. 7b).”

6. Because the double Rpd3-2SA Ash1-2SA mutant does not show a further reduction

of H3 acetylation, the authors conclude that Rdp3-2SA may play a more prominent role than Ash1-2SA (lines 384-386). However, the levels of acetylation of both single mutants are similar in fig. 7C. Therefore, I wonder why the authors reach or propose this conclusion?

We thank the reviewer for this point. As the histone acetylation levels in Rpd3-2SA were slightly lower than Ash1-2SA (Fig. 7c), that is why we proposed Rdp3-2SA may play a more prominent role than Ash1-2SA. To prevent any confusion, we removed this sentence from the result section.

Line 390: Deleted ", suggesting that Rpd3-2SA may play a more predominant role than Ash1-2SA".

7. The type of mammalian cells used in this work should be stated in the main text.

We thank the reviewer for this point. We used hepatocellular carcinoma HepG2 cells in this study. We added this information in the result section as requested.

Lines 458-460: Changed "Knockdown of PRKACA and PRKACB with short hairpin RNA (shRNA) decreased the global levels of H3K9ac and H3K14ac (Fig. 8a)" to "Knockdown of PRKACA and PRKACB with short hairpin RNA (shRNA) decreased the global levels of H3K9ac and H3K14ac in hepatocellular carcinoma HepG2 cells (Fig. 8a)".

8. In Fig. 8b the blot shown appears to demonstrate that HDAC8 knockdown leads to a complete disappearance. This is not discussed at all.

We thank the reviewer for this point. We carefully checked the blots and found that we loaded less HDAC8-knockdown samples when compared with other lanes as indicated by reduced amount of histone H3 (Original Fig. 8b, lane 5 vs lane 1). Based on our quantification, knockdown of HDAC8 led to a reduction but not complete disappearance of H3 acetylation (Fig. 8b). For the sake of clarity, we re-performed the immunoblots and observed slight reduction of H3 acetylation in HDAC8-kncokdown cells (Fig. 8b). The slight reduction of H3 acetylation could be explained by the fact that HDAC8 may indirectly affect histone acetylation by deacetylating other non-histone proteins.

9. Fig 8H Nucleosomes and not nuclesomes.

We thank the reviewer for this point. We made changes as requested.

Point-by-point response to the referees' comments

REVIEWER COMMENTS
Our responses are in blue.

Reviewer #1 (Remarks to the Author):

The Authors did an excellent job improving the manuscript according to the suggestions provided in the first round of evaluation. I think this is a nice piece of work, investigating the role of Rpd3 phosphorylation in yeast and of the mammalian ortholog HDAC1 in the metabolic adaptation. The results have relevant implications in the regulation of metabolic pathways in mammals, as nicely shown with the experiments performed in HepG2 cells.

As far as I am concerned, I have only a couple of doubts, and I would like to ask the Authors to make a final effort to address and improve the manuscript.

We thank the reviewer for recognition of our work. We explained why we chose IDP2 in our RT-qPCR analysis. We also performed additional experiments to address the effect of PKA-HDAC1 axis on *ACLY* and *SLC25A1* transcription. The point-by-point answers to all the questions raised by the reviewer are enumerated below.

1. In Figure 7h and 7i they measured the mRNA levels and histone acetylation of some genes supposedly involved in TCA cycle in yeast. However, among these genes they tested IDP2, which encodes the cytosolic NADP-specific isocitrate dehydrogenase and not the mitochondrial isocitrate dehydrogenase, the one actually involved in TCA cycle. Can you, please, explain?

We thank the reviewer for this point. IDP2 is capable of catalyzing NADP-dependent oxidative decarboxylation of isocitrate to form α-ketoglutarate in *Saccharomyces cerevisiae*. Although IDP2 is not a mitochondrial isocitrate dehydrogenase, it has been suggested to provide α-ketoglutarate both for TCA cycle carbon flux and for cytosolic glutamate synthesis (PMID: 8099357). Thus, IDP2 was categorized into TCA cycle and related pathways in several references (PMID: 18629096; PMID: 38004686).

2. As suggested after the first round of evaluation, the Authors tried to detect the nuclear form of ACLY using immunofluorescence microscopy. They reported that 8-Bromo-cAMP treatment "slightly increased nuclear localization of ACLY in both Flag-HDAC1 and Flag-HDAC1-S434A HepG2 cells (Supplementary Fig. 12a)", however they did not quantify the change. According to the images in Supplementary Fig. 12a, although

there seems to be a slight increase, the difference may not be significant as the difference is not striking. An alternative to prove the importance of providing the substrate acetyl-CoA for histone acetylation could be to measure the expression of ACLY and of the mitochondrial citrate transporter SLC25A1. This could be done quickly by qPCR with RNA the Authors have already used to quantify the levels of other mRNAs reported in supplementary Figure 10b and 10c.

We thank the reviewer for this suggestion. In the revised manuscript, we quantified the images in Supplementary Fig. 12a. It revealed that 8-Bromo-cAMP treatment slightly but significantly increased the nuclear localization of ACLY in both Flag-HDAC1 and Flag-HDAC1-S434A HepG2 cells (Supplementary Fig. 12a). This modest yet significant increase in nuclear ACLY likely provides acetyl-CoA for histone acetylation.

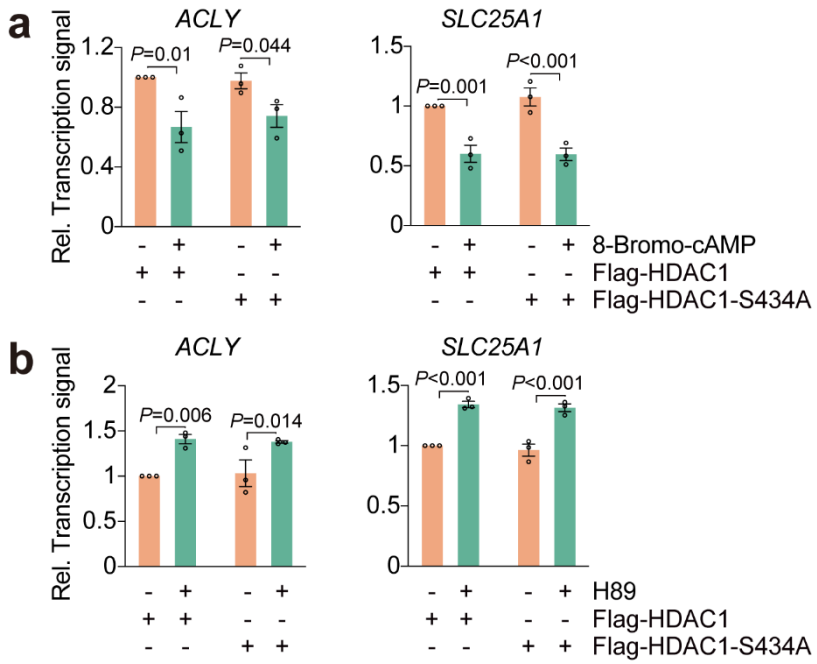
As suggested by the reviewer, we examined the effect of PKA-HDAC1 axis on the expression of ACLY and of the mitochondrial citrate transporter SLC25A1 with RNA used in Supplementary Figure 10b and 10c. The results showed that 8-Bromo-cAMP decreased the transcription of *ACLY* and *SLC25A1* in both HDAC1-Flag and HDAC1-S434A-Flag mutant cells (**See Rebuttal Letter Fig. 1a**). In contrast, PKA inhibitor H89 increased the transcription of *ACLY* and *SLC25A1* in both HDAC1-Flag and HDAC1-S434A-Flag mutant cells (**See Rebuttal Letter Fig. 1b**). These data suggest that PKA repressed *ACLY* transcription while promoting its nuclear localization. Although 8-Bromo-cAMP had opposing effects on *ACLY* transcription and its nuclear localization, our findings suggest that these regulatory effects are not mediated through PKA-dependent phosphorylation of HDAC1. PKA may exert these effects by phosphorylating other proteins, including ACLY, which merits further investigation in the next paper.

The following are our interpretations of these results and response to the reviewer's comment about providing the substrate acetyl-CoA for histone acetylation:

1. The increased histone acetylation at the promoter regions of TCA genes may be attributed to the concerted actions of PKA. Specifically, the modest yet significant increase in nuclear ACLY likely provides acetyl-CoA for histone acetylation. Additionally, PKA-mediated repression of HDAC1's deacetylase activity further enhances histone acetylation. These two mechanisms likely contribute to the elevated histone acetylation observed at the promoters of TCA cycle genes.
2. ACLY is not the sole source of acetyl-CoA for histone acetylation. There are other possible sources of acetyl-CoA for histone acetylation. For example, pyruvate dehydrogenase 1α (PDHE1α) has been reported to provide acetyl-CoA for chromatin acetylation in response to DNA damage (PMID: 37735618).
3. 8-Bromo-cAMP treatment increased histone acetylation in Flag-HDAC1 cells and mutation of HDAC1-S434A significantly reduced 8-Bromo-cAMP-induced increase of histone acetylation (Supplementary Fig. 12b), suggesting that PKA induced histone acetylation primarily by repressing HDAC1 activity.

Together, our data revealed a new mechanism for how PKA increased histone

acetylation by phosphorylating and inhibiting HDAC1. Nonetheless, HDAC1 may not be the sole target for PKA to promote histone acetylation. If you have any better suggestions, we are happy to change the manuscript.



Rebuttal Letter Fig. 1. RT-qPCR analysis of *ACLY* and *SLC25A1* transcription in shHDAC1 HepG2 cells that ectopically expressed Flag-HDAC1 and Flag-HDAC1-S434A when treated with 8-Bromo-cAMP (**a**) or H89 (**b**). Data represent means ± SE; n=3 biological independent experiments; two-way ANOVA with Tukey's multiple comparisons tests were used for statistical analysis.

Line 629: Changed "It revealed that 8-Bromo-cAMP treatment slightly increased the nuclear localization of ACLY in both HDAC1-Flag and HDAC1-S434A-Flag mutant (Supplementary Fig. 12a)." to "It revealed that 8-Bromo-cAMP treatment slightly but significantly increased the nuclear localization of ACLY in both HDAC1-Flag and HDAC1-S434A-Flag mutant (Supplementary Fig. 12a). This modest yet significant increase in nuclear ACLY likely provides acetyl-CoA for histone acetylation."