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DELLA-mediated gene repression is maintained by chromatin modification in rice

Junjie Li, Qi Li, Wentao Wang, Xinran Zhang, Chen Chu, Xintian Tang, Bo Zhu, Lizhong Xiong, Yu Zhao, and Dao-Xiu Zhou
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Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision **12th May 2023**

Dear Prof. Zhou,

Thank you again for the submission of your manuscript entitled "Chromatin mechanism of DELLA-mediated gene repression and GA-induced gene activation in rice" (EMBOJ-2023-114220) to our editorial office. We have now received reports from two referees, which I copy below.

As you can see from their comments, both referees agreed that your use of SLENDER RICE1 to address the role of epigenetic modification in gibberellin signalling is a sensible approach; they also state that the topic is both relevant and timely. However, alongside such positive comments, the referees point out areas of the manuscript that will require further strengthening before the study can be published in The EMBO Journal. These comments include questions over the relationship between GA signalling and PRC2 function, the mechanism through which SLR1 guides PRC2 to relevant loci, and a wide range of technical concerns. I agree with the referees that the work would benefit from an expanded genetic analysis, possibly focusing on the quantification of those physiological resopnses which are most sensitive to GA.

However, based on the overall interest expressed by the referees, I would like to invite you to address the comments of all referees in a revised version of the manuscript. I should add that it is The EMBO Journal policy to allow only a single major round of revision and that it is therefore important to resolve the main concerns at this stage. I believe the concerns of the referees are reasonable and addressable, but please contact me if you have any questions, need further input on the referee comments or if you anticipate any problems in addressing any of their points. I would be happy to talk all this over via Zoom. Please, follow the instructions below when preparing your manuscript for resubmission.

I would also like to point out that as a matter of policy, competing manuscripts published during this period will not be taken into consideration in our assessment of the novelty presented by your study ("scooping" protection). We have extended this 'scooping protection policy' beyond the usual 3 month revision timeline to cover the period required for a full revision to address the essential experimental issues. Please contact me if you see a paper with related content published elsewhere to discuss the appropriate course of action.

When preparing your letter of response to the referees' comments, please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

Again, please contact me at any time during revision if you need any help or have further questions.

Thank you very much again for the opportunity to consider your work for publication. I look forward to your revision.

Best regards,

William Teale

William Teale, Ph.D.
Editor
The EMBO Journal

When submitting your revised manuscript, please carefully review the instructions below and include the following items:

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point response to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/Author Checklist%20-%20EMBO%20J-1561436015657.xlsx). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript.

6) We require a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public (see https://www.embopress.org/page/journal/14602075/authorguide#datadeposition). If no data deposition in external databases is needed for this paper, please then state in this section: This study includes no data deposited in external repositories. Note that the Data Availability Section is restricted to new primary data that are part of this study.

Note - All links should resolve to a page where the data can be accessed.

7) When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
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Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

8) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.).

9) We would also encourage you to include the source data for figure panels that show essential data. Numerical data can be provided as individual .xls or .csv files (including a tab describing the data). For 'blots' or microscopy, uncropped images should be submitted (using a zip archive or a single pdf per main figure if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available at .

10) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online (see examples in https://www.embopress.org/doi/10.15252/embj.201695874). A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc. in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: .

- Additional Tables/Datasets should be labelled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

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Further instructions for preparing your revised manuscript:

Please make sure you upload a letter of response to the referees' comments together with the revised manuscript.

Please also check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:

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- individual production quality figure files (one file per figure)
- a complete author checklist, which you can download from our author guidelines (https://www.embopress.org/page/journal/14602075/authorguide).
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We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (10th Aug 2023). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

https://emboj.msubmit.net/cgi-bin/main.plex

Referee #1:

The phytohormone GA plays the important roles in regulating plant growth and adaptation to multiple environmental cues. However, although the GA-GID1-DELLA signaling has been well demonstrated, the mechanisms underlying GA-DELLA regulatory system mediated gene expression still remain unclear. In this manuscript, the authors reported that the rice DELLA protein SLR1 could form a protein complex with PRC2 and HDA702 to regulate target gene by chromatin modification. This topic of this manuscript is interest for researchers follow trends and development in understanding molecular mechanisms of GA action and its role roles in regulating plant development and growth. However, there are deficiencies in experimental analysis that require substantiation before the manuscript can be acceptable.

A lot of microarrays and RNA-seq experiments have revealed that the GA-DELLA regulatory module either activates or represses target genes expression. DELLA protein usually interacts and interfere with its-interacting transcription factors' (for example, PIF3 and GRF4) activities, and consequently inhibits the target gene expression. In this manuscript, the authors reported a new mechanism of GA-induced gene activation through chromatin modification. Although SLR1 belongs to GRAS family, there is no solid evidence that SLR1 (as well as GAI, RGA and RGLs in Arabidopsis) bind to the DNA fragments. In fact, previous studies have shown PRC2-mediated repression of target gene by sequence-specific DNA-binding factors. The key question is which (how) SLR1-interacting transcription factors involved in GA-GID1-mediated responses?

The authors found that the rice DELLA protein SLR1 is required to maintain high levels of H3K27me3, and that SLR1 was also found to interact directly with SDG711, SDG718, EMF2b, FIE2 and MSI1, does SLR1 affect the formation of PRC2 complex? Or the protein stability of these components of PRC2 complex?

In Fig. 6, the authors showed that the histone modification levels of H3K9ac increased (or H3K27me3 decreased) with an increasing time of exposure GA treatments. However, although it is known that GA promotes plant growth by GA receptor GID1-mediated degradation of the DELLA destruction, the authors should add additional experiments using GA-insensitive mutant including null gid1 mutant and gain-of-function slr1-d mutant (such as slr1-d6).

To confirm chromatin mechanism underlying DELLA-mediated gene repression or GA-GID1-induced gene activation, the authors should provide the solid genetic evidences. For example, in Fig. 5, the authors should compare the chromatin modification levels of GA-regulated genes between WT, slr1, fie2, slr1/fie2 double mutant and slr-6/fie2 double mutant with and

without GA treatments.

To investigate of epigenetic mechanism DELLA-mediated gene repression, it will be better that authors choose one of physiological responses that is largely sensitive to GA (for example, second leaf sheath growth, culm elongation, or seed germination),

There is a mistake in Fig. 1F, EMF2b, not EMF2

Referee #2:

In this manuscript Li et al describe new mechanistic insight into the repressive mode of action of the rice DELLA orthologue SLR1. They report that SLR1 interacts with components of the polycomb repressive complex 2 (PRC2), and recruits them to its targets, where they promote repression by H3K27me3 chromatin modifications. SLR1 also interacts with a histone deacetylase that promotes SLR1-dependent deacetylation of its target genes. After gibberellic acid (GA)-treatment, SLR1 protein is degraded and the chromatin modifiers are lost from selected target loci. This manuscript provides an exciting and as far as I can see previously unexplored link between GA signalling/DELLA protein action and gene repression by PRC2. The manuscript is well structured and the data seem solid and the conclusions well supported. I have, a number of concerns and suggestions for improvement.
(1) The potential link between PRC2 and GA signaling is exciting. Have the authors or anyone else checked whether mutants of PRC2 have altered GA responses? This would be an important step towards confirming the suggested mode of action.
(2) How many of the PRC2 targets (as defined by enrichment of H3K27me3 or by direct binding of PRC2 components) show altered H3K27me3 and differential expression in slr1 mutants or after GA-treatment? In other words, can the authors define what fraction of PRC2 targets require SLR1?
(3) Are the targets characterized in Fig. 5-7 directly targeted by SLR1? This can be investigated by ChIP-qPCR with the antibody they generated or the tagged SLR1 line. The model states that SLR1 controls a subset of PRC2 targets. An important control for the ChIP-qPCR would be to include one classic PRC2 target whose regulation is independent of SLR1. This might clarify the mechanism.
(4) Given the authors performed ChIP-seq of H3K27me3 in slr1 mutants and after GA-treatment, a quantitative comparative analysis of the peaks should be added to clarify the dynamics of interactions.
(5) RNAseq of GA-treated plants was performed at 1 h after induction. In contrast, the ChIP-seq was performed after 6 h. This makes comparison difficult. In addition, the authors themselves state that they find an unexpectedly low number of differentially expression genes. Given the crucial role of this experiment, the manuscript would benefit greatly from transcriptomic data at 6h after GA induction.
(6) Figs. 2a, 6a: I find it very surprising that there is such a large global effect on these two modifications, after all, GA-regulated genes make up only a small fraction of genes that are targeted by H3K27me3 and H3K9ac. How was the quantification done? Was the signal normalized to global H3 signal? Do the authors have an explanation for the magnitude of effect?
(7) Fig 7b-c: Protein levels from overexpression constructs were analyzed for FIE2-HA and HSA702-HA. Can the authors rule out that the overexpression obliterates regulation of the endogenous protein (at the level of transcription, but possibly also protein synthesis or turnover)?
(8) PCA analysis of the RNAseq in slr1 would be helpful in assessing the quality of the data. The GA gene expression heatmap in Fig. 3b shows quite a bit of variation between replicates. (analogous to Fig. S8a - actually the third GA-1h replicate is quite divers from the others, any thoughts on this?).
Minor comments:
The manuscript would benefit from language editing (grammar and spelling). E. g. l. 84 "depednent", l. 100 "singling", l. 246 "sequestrating", Fig. 3 legend title: "reppressed"
l. 186: "largely up-regulated", unclear what is meant by largely. To a strong extent? A small trend in some? Please clarify.
l. 254: please clarify what is meant by "higher hierarchical transcriptional repressors"
l. 276: "first event": tone down statement. Only two chromatin modifications were tested.
The last chapter of the results is very long. Consider to break in two or three shorter sections.
Fig. 3c: top panel: grey line poorly visible, please change color.
Fig. 6c: Y axis label missing

Reply to reviewers' comments

Referee #1:

The phytohormone GA plays the important roles in regulating plant growth and adaptation to multiple environmental cues. However, although the GA-GID1-DELLA signaling has been well demonstrated, the mechanisms underlying GA-DELLA regulatory system mediated gene expression still remain unclear. In this manuscript, the authors reported that the rice DELLA protein SLR1 could form a protein complex with PRC2 and HDA702 to regulate target gene by chromatin modification. This topic of this manuscript is interest for researchers follow trends and development in understanding molecular mechanisms of GA action and its role roles in regulating plant development and growth. However, there are deficiencies in experimental analysis that require substantiation before the manuscript can be acceptable.

1. A lot of microarrays and RNA-seq experiments have revealed that the GA-DELLA regulatory module either activates or represses target genes expression. DELLA protein usually interacts and interfere with its-interacting transcription factors' (for example, PIF3 and GRF4) activities, and consequently inhibits the target gene expression. In this manuscript, the authors reported a new mechanism of GA-induced gene activation through chromatin modification. Although SLR1 belongs to GRAS family, there is no solid evidence that SLR1 (as well as GAI, RGA and RGLs in Arabidopsis) bind to the DNA fragments. In fact, previous studies have shown PRC2-mediated repression of target gene by sequence-specific DNA-binding factors. The key question is which (how) SLR1-interacting transcription factors involved in GA-GID1-mediated responses?

Thanks for the comments. It is indeed important to understand how SLR1-PRC2 target downstream genes. Although different mechanisms may be involved in the process (as we have discussed in text), recruitment by DNA-binding transcription factors represents a more plausible pathway. To test this hypothesis, we analyzed by bioinformatics the promoters of the 397 genes that showed up-regulation of expression and downregulation of H3K27me3 in *slr1* plants. We found that these genes could be potentially recognized or bound by members of ZnF, MYB, NAC, WRKY, and bZIP transcription factor families (Fig S7A). Among the ZnF family, YABBY4 (YAB4) was previously shown to interact with SLR1 to repress GA -responsive genes in rice (Yang, Ma et al., 2016). In addition, YAB4-binding site could be identified in several tested SLR1 target genes (Fig S7B). We therefore analyzed *yab4* mutant and *YAB4-Flag* overexpression plants (previously produced in the lab for another project) to test YAB4-, SLR1- and PRC2 (EMF2b) -binding to *SLR1* downstream genes (*PLA1* and *EXPB8*). Analysis of the *YAB4-Flag* plants with anti-Flag ChIP-PCR assays indicated that the two tested SLR1 downstream genes (*PLA1* and *EXPB8*) were bound by YAB4 (Fig S7C), that the *yab4* mutation reduced the levels of SLR1 and EMF2b-binding to the targets (Fig S7D). The data shown in Fig S7 are commented in the text.

2. The authors found that the rice DELLA protein SLR1 is required to maintain high levels of H3K27me3, and that SLR1 was also found to interact directly with SDG711, SDG718, EMF2b, FIE2 and MSI1, does SLR1 affect the formation of PRC2 complex? Or the protein stability of these components of PRC2 complex? does SLR1 affect the formation of PRC2 complex?

We had tested the stability of PRC2 component (EMF2b) as well as HDA702 in *slr1* plants as well as in GA-treated wild type plants (please see Fig 7A). The *slr1* mutation or GA treatment did not alter the endogenous protein levels of EMF2b and HDA702.

3. In Fig. 6, the authors showed that the histone modification levels of H3K9ac increased (or H3K27me3 decreased) with an increasing time of exposure GA treatments. However, although it is known that GA promotes plant growth by GA receptor GID1-mediated degradation of the DELLA destruction, the authors should add additional experiments using GA-insensitive mutant including null gid1 mutant and gain-of-function slr1-d mutant (such as slr1-d6).

Thanks for the advice. We have obtained the *gid1* and the *slr1-d5* (gain-of-function) mutants from other labs and analyzed the H3K9ac and H3K27me3 levels in the mutant plants treated with or without GA at different time points. The results shown in Fig S8C, D indicate that the histone modification levels were unchanged in *gid1* plants treated or untreated with GA. In *slr1-d5* plants, GA-treatment induced about 20% increase of H3K9ac and decrease of H3K27me3 6h after the treatment, which is less important and later than in WT plants. The data confirm that GA-signaling leads to alteration of the histone modifications.

4. To confirm chromatin mechanism underlying DELLA-mediated gene repression or GA-GID1-induced gene activation, the authors should provide the solid genetic evidences. For example, in Fig.5, the authors should compare the chromatin modification levels of GA-regulated genes between WT, slr1, fie2, slr1/fie2 double mutant and slr-6/fie2 double mutant with and without GA treatments.

We agree that comparing effects of *slr1* and *fie2* single and double mutations on chromatin modifications would strengthen the conclusion. As the *slr1* is infertile (the mutant is maintained at heterozygous state) and the rice *fie2* mutation is lethal (which already eliminates most H3K27me3), it is impossible in practical to obtain such double mutations. In addition, H3K27me3 is already low or absent in *prc2* mutants, an effect of GA-treatment to further reduce H3K27me3 would be hard to detect. Furthermore, making double mutations (by genetic crosses) in rice needs at least two years.

5. To investigate of epigenetic mechanism DELLA-mediated gene repression, it will be better that authors choose one of physiological responses that is largely sensitive to GA (for example, second leaf sheath growth, culm elongation, or seed germination).

Thanks for the suggestion. We have tested the second leaf sheath growth in GA-treated WT and *emf2*b RNAi plants published previously (Tan, Wang et al., 2022). The data shown in Fig S8E indicated that the *emf2b* RNAi plants are hypersensitive to GA compared with WT, which is consistent with the repression of GA-signaling by PRC2.

There is a mistake in Fig. 1F, EMF2b, not EMF2

Changed (Fig. 1F)

Referee #2:

In this manuscript Li et al describe new mechanistic insight into the repressive mode of action of the rice DELLA orthologue SLR1. They report that SLR1 interacts with components of the polycomb repressive complex 2 (PRC2), and recruits them to its targets, where they promote repression by H3K27me3 chromatin modifications. SLR1 also interacts with a histone deacetylase that promotes SLR1-dependent deacetylation of its target genes. After gibberellic acid (GA)-treatment, SLR1 protein is degraded and the chromatin modifiers are lost from selected target loci. This manuscript provides an exciting and as far as I can see previously unexplored link between GA signaling/DELLA protein action and gene repression by PRC2. The manuscript is well structured and the data seem solid and the conclusions well supported. I have, a number of concerns and suggestions for improvement.

(1) The potential link between PRC2 and GA signaling is exciting. Have the authors or anyone else checked whether mutants of PRC2 have altered GA responses? This would be an important step towards confirming the suggested mode of action.

Thanks for the comments. Actually this point was also raised by reviewer 1. We have tested the second leaf sheath growth in GA-treated WT and *emf2*b RNAi plants. The data shown in Fig S8E indicated that the *emf2b* RNAi plants are hypersensitive to GA compared with WT, which is consistent with the repression of GA-signaling by PRC2.

(2) How many of the PRC2 targets (as defined by enrichment of H3K27me3 or by direct binding of PRC2 components) show altered H3K27me3 and differential expression in slr1 mutants or after GA-treatment? In other words, can the authors define what fraction of PRC2 targets require SLR1?

Thanks for the comments. Because the *slr1* mutation resulted in decrease of H3K27me3 in 3,615 of the 12,684 total marked genes in the genome, about 28.5% of the PRC2 targets would require SLR1. We have added a sentence at line 126.

(3) Are the targets characterized in Fig. 5-7 directly targeted by SLR1? This can be investigated by ChIP-qPCR with the antibody they generated or the tagged SLR1 line. The model states that SLR1 controls a subset of PRC2 targets. An important control for the ChIP-qPCR would be to include one classic PRC2 target whose regulation is independent of SLR1. This might clarify the mechanism.

We had shown that SLR1 associated directly with the target genes tested in Fig 5-7 (please see Fig 5C, ChIP-PCR with anti-SLR1).

As advised, we have included a SLR1-independnet PRC2 target, *MPS,* in the analysis. MPS gene encodes a MYB factor (Schmidt, Schippers et al., 2013). This gene is modified by H3K27me3, and is bound by EMF2b, but not by SLR1 (Figure 5C, D; Fig S6C). GA-treatment or the *slr1* mutation did not change the level of H3K27me3 or expression of the gene (Fig S6C). The new data supported that SLR1 is required for a subset of PRC2 targets and are commented in the text.

(4) Given the authors performed ChIP-seq of H3K27me3 in slr1 mutants and after GA-treatment, a quantitative comparative analysis of the peaks should be added to clarify the dynamics of interactions.

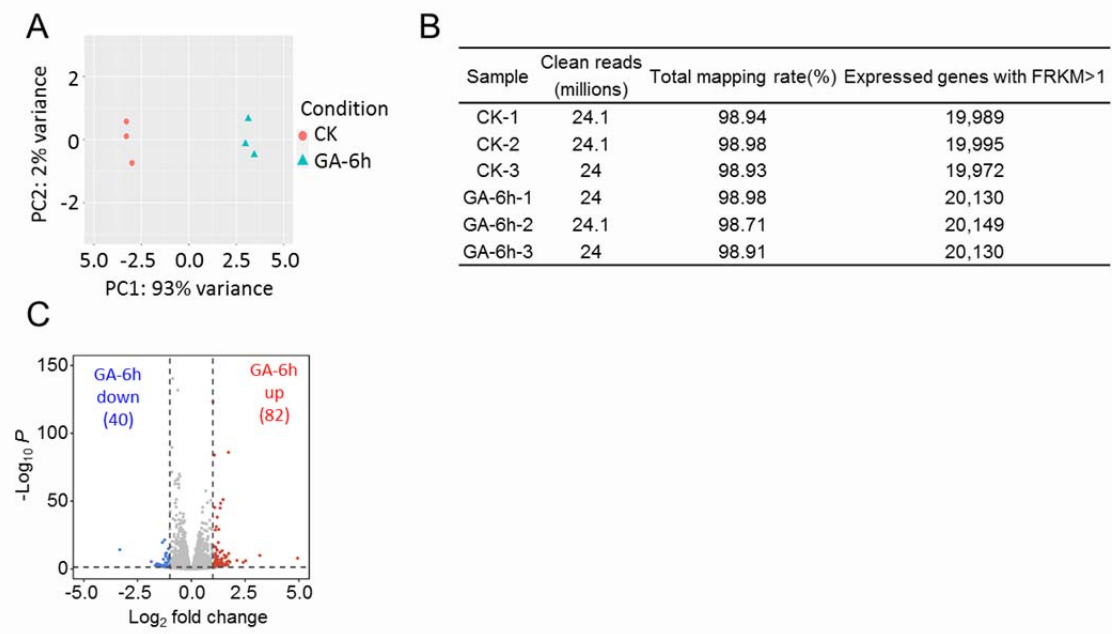
Thanks for the suggestion. We have included metaplots of the H3K27me3 ChIP-seq peak levels of GA treated and untreated WT plants and *slr1* mutants in Fig S10D. The comparison is commented in the MS text.

(5) RNAseq of GA-treated plants was performed at 1 h after induction. In contrast, the ChIP-seq was performed after 6 h. This makes comparison difficult. In addition, the authors themselves state that they find an unexpectedly low number of differentially expression genes. Given the crucial role of this experiment, the manuscript would benefit greatly from transcriptomic data at 6h after GA induction.

Thanks for the comments. The reason that we chose to analyze transcriptome at 1h of the GA treatment was based on the observation that the increase of H3K9ac (that is tightly associated with gene activation) clearly occurred early during GA treatment, while the decrease of H3K27me3 (that marks gene repression state) lagged behind (3-6h after the treatment (Fig 6)). In addition, investigating transcriptome at an early time point of GA treatment would catch up better genes that are directly activated by GA signaling.

We have followed your suggestion and performed RNA-seq of plants after 6h GA treatment. The RNA-seq repeats are of good quality, but unfortunately very few differentially expressed genes were identified (please see below), hampering further analysis.

The analysis of transcriptomes at 1h GA shown in Fig S9D corroborates the main conclusion.



Sample	Clean reads (millions)	Total mapping rate(%)	Expressed genes with FRKM>1
CK-1	24.1	98.94	19,989
CK-2	24.1	98.98	19,995
CK-3	24	98.93	19,972
GA-6h-1	24	98.98	20,130
GA-6h-2	24.1	98.71	20,149
GA-6h-3	24	98.91	20,130

Figure 1. RNA-seq analysis of rice seedlings treated with or without GA for 6h.

A. Repeatability of RNA-seq data revealed by PCA. **B.** Summary of RNA-seq raw data. **C.** Average read count plots of gene expression induced 6 h GA treatment. The x axis represents log2 FPKM fold change (treated /untreated) and the y axis represents average gene expression level (FPKM). Significant (P<0.05 and 2-fold change) up-regulated and down-regulated gene numbers are indicated.

(6) **Figs. 2a, 6a**: I find it very surprising that there is such a large global effect on these two modifications, after all, GA-regulated genes make up only a small fraction of genes that are targeted by H3K27me3 and H3K9ac. How was the quantification done? Was the signal normalized to global H3 signal? Do the authors have an explanation for the magnitude of effect?

The experiments were independently repeated for at least 3 times. According to ChIP-seq data, SLR1 is required for H3K27me3 of about 28.5% of the PRC2 targets. The decrease (about 50%) of H3K27me3 (Fig 2a) in the *slr1* plants relative to WT, which was confirmed by the difference of the ChIP-seq H3K27me3 peak levels between *slr1* and WT CK plants (Fig S10D), may be also partly due to developmental defects of the *slr1* mutant. We have discussed the point in the text.

The large effects of GA on the histone modifications are observable 6h - 12h after the treatment (for H3K27me3). One possible explanation would be that after 6h-12h treatment GA stimulated cell proliferation, during which H3K27me3 was not maintained or diluted, while H3K9ac is accumulated, as histone acetylation usually associates with cell proliferation, as observed in yeast and mammalian cells.

(7) **Fig 7b-c**: Protein levels from overexpression constructs were analyzed for FIE2-HA and HSA702-HA. Can the authors rule out that the overexpression obliterates regulation of the endogenous protein (at the level of transcription, but possibly also protein synthesis or turnover)?

We have obtained HDA702 antibody from another lab and tested endogenous HDA702 protein levels in WT plants treated by GA. Because we already tested the PRC2 protein levels with anti-EMF2b antibody in in WT plants treated with GA, we deleted the data of GA-treated OE-FIE2 plants.

(8) **PCA analysis of** the RNAseq in slr1 would be helpful in assessing the quality of the data. The GA gene expression heatmap in Fig. 3b shows quite a bit of variation between replicates. (analogous to Fig. S8a - actually the third GA-1h replicate is quite divers from the others, any thoughts on this?).

We have put back the correlation coefficients of the RNA-seq replicates and rescaled the axis of the PCA (Fig S9A). The replicates are of good quality. Fig 3b heatmap shows individual genes (not reflecting statistical situation).

Minor comments:

The manuscript would benefit from language editing (grammar and spelling).

E. g. l. 84 "depednent", l. 100 "singling", l. 246 "sequestrating", Fig. 3 legend title: "reppressed"

Corrected (Line 85; 101; 291; 738).

l. 186: "largely up-regulated", unclear what is meant by largely. To a strong extent? A small trend in some? Please clarify.

Changed to “2-4 folds” (Line 190).

l. 254: please clarify what is meant by "higher hierarchical transcriptional repressors"

Reworded to “master transcriptional repressors” (Line 299).

l. 276: "first event": tone down statement. Only two chromatin modifications were tested.

Reworded to “H3K9ac increased prior to H3K27me3 decline” (Line 321).

The last chapter of the results is very long. Consider to break in two or three shorter sections.

Divided into 3 sections (Line 181-288).

Fig. 3c: top panel: grey line poorly visible, please change color.

Changed (Fig. 3c).

Fig. 6c: Y axis label missing

Added (Fig. 6c).

Reference

Schmidt R, Schippers JHM, Mieulet D, Obata T, Fernie AR, Guiderdoni E, Mueller-Roeber B (2013) MULTIPASS, a rice R2R3-type MYB transcription factor, regulates adaptive growth by integrating multiple hormonal pathways. *Plant Journal* 76: 258-273

Tan FQ, Wang W, Li J, Lu Y, Zhu B, Hu F, Li Q, Zhao Y, Zhou DX (2022) A coiled-coil protein associates Polycomb Repressive Complex 2 with KNOX/BELL transcription factors to maintain silencing of cell differentiation-promoting genes in the shoot apex. *Plant Cell* 34: 2969-2988

Yang C, Ma YM, Li JX (2016) The rice YABBY4 gene regulates plant growth and development through modulating the gibberellin pathway. *J Exp Bot* 67: 5545-5556

Dear Dao-Xiu,

We have now received re-review reports from two of the three referees that initially appraised your manuscript. As you will see below, you have addressed their concerns satisfactorily. Before I can finally accept the manuscript though, I have noticed some small editorial points that remain and need to be addressed. In this regard would you please:

remove track changes from the final .doc manuscript text,
include an ORCID identifier for Dr Zhao,
add up to five keywords,
use 10 authors + et al. for Xiao et al. (2017) in the reference section,
rename the conflict of interest section the 'Disclosure and competing interests statement'
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upload figures in separate files,
rename Table EV4 as Dataset EV1, upload the legend in a separate sheet of the Excel file and modify callouts in the text as appropriate,
include a Table of Contents with page numbers in Appendix 1,
upload the synopsis image as a separate file,
ensure datasets GSE226812 and PXD040644 are publicly available,
upload EV tables as individual files, there should be seven EV Tables in total, and
remove appendix figure legends and EV Table legends from the main manuscript file.

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Best wishes,

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We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (8th Nov 2023). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

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Referee #1:

In this revised version of the manuscript, the authors have added additional experiments and answered the most of questions which I addressed, it now is acceptable for publication.

Referee #2:

The authors have made a thorough effort and addressed my previous concerns in their revised manuscript.

Reply to reviewers' comments

Referee #1:

In this revised version of the manuscript, the authors have added additional experiments and answered the most of questions which I addressed, it now is acceptable for publication.

Response: Thank you for your support.

Referee #2:

The authors have made a thorough effort and addressed my previous concerns in their revised manuscript.

Response: Thank you for your support.

2nd Revision - Editorial Decision **21st Aug 2023**

Dear Dao,

I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal.

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Please note that it is EMBO Journal policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper. If you do NOT want this, you will need to inform the Editorial Office via email immediately. More information is available here: https://www.embopress.org/page/journal/14602075/authorguide#transparentprocess

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Best wishes,

William

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Reporting Checklist for Life Science Articles (updated January

This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your

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The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
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- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

2. Captions

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

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
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- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
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