The Chromatin-associated Protein PWO1 Interacts with Plant Nuclear Lamin-like Components to Regulate Nuclear Size

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REPORT: (The report shows the major requests for revision and author responses. Minor comments for revision and miscellaneous correspondence are not included. The original format may not be reflected in this compilation, but the reviewer comments and author responses are not edited, except to correct minor typographical or spelling errors that could be a source of ambiguity.)

TPC2018-00663-RA 1st Editorial decision – *revision requested* Oct. 12, 2018

We have received reviews of your manuscript entitled "The chromatin-associated protein PWWP INTERACTOR OF POLYCOMBS (PWO1) regulates nuclear morphology in Arabidopsis." Thank you for submitting your best work to *The Plant Cell*. The editorial board agrees that the work you describe is substantive, falls within the scope of the journal, and may become acceptable for publication pending revision, and potential re-review.

We ask you to pay attention to the following points in preparing your revision. As you will see from the detailed comments of the reviewers, they both find the data interesting, adding to our knowledge about the chromatin organization in Arabidopsis. However, both reviewers were not content with the resolution of the localization images, and Reviewer 2 asks for more details about the interaction data. Also, the manuscript preparation needs more care. Please consider the suggestions for more precise terminology, include the additional references, correct the legends and answer the individual questions.

---------- Reviewer comments:

[Reviewer comments shown below along with author responses]

TPC2018-00663-RAR1 1st Revision received Dec. 21, 2018

Reviewer comments and **author responses**:

We thank the editors and reviewers for their constructive criticism and appreciation of our work. We have addressed all the points raised, please see below and modified text and figures.

Reviewer #1:

The manuscript provides novel and very interesting information about the links between PWO1 and CRWN1/NMCP1, a constitutive protein of the plant nuclear lamina, involving this structure in chromatin regulation and opens the field for the understanding of the roles of CRWN proteins in gene regulation.

Minor modifications:

The title is neither descriptive of the whole work nor attractive. It should mention the novel link between PWO1 and CRWN1 proteins and its implications for the role of the plant lamina in chromatin regulation.

We agree that we kept the title rather general. We now modified the title to "The chromatin-associated protein PWO1 interacts with plant nuclear lamin-like components to regulate nuclear size".

The localization of PWO1 in A. thaliana is not clearly resolved with the technology used, being more in a nuclear network than in foci. May be the use of STED or similar high-resolution techniques may help to clarify this point.

We have used a super-resolution technique (Zeiss LSM880 with Airyscan) that improved the image resolution (see figure 2 and methods), but could not clearly resolve the issue raised by the reviewer, Still, root meristematic nuclei show a more focal-like PWO1 pattern than nuclei of differentiated cells, but it cannot be excluded that foci are connected. There is no clear co-localization with the nuclear lamina of PWO1-GFP. As PWO1-GFP shows only weak GFP signal (but complements the phenotypes) other methods such as SIM were not successful and STED would likely not be feasible with this line. Overall, we believe that the new analyses have clearly improved the resolution of the images.

Figures

Figure 1. The legends of the different parts of the figure are changed, please reorder.

Thank you for pointing this out – we have corrected the legend.

Figures 2 and S6. The distribution of PWO1 in meristematic cells looks like a network, not like foci. A technique with more resolution is needed.

See above.

Fig S2. How do the authors explain the internal nuclear labelling of CRWN1-GFP and CRWN1-mCh?

We had addressed this in the text, line 202 ff.: "Of note, in our hands, CRWN1 protein transiently expressed in N. benthamiana leaves showed not only NE-localization, but was also present uniformly in the nucleoplasm and caused nuclear membrane deformations, as reported in (Goto et al., 2014; Graumann, 2014b)." The internal nuclear labelling is probably a result of CRWN1 overexpression, but indicates functionality of the used construct.

References: Graumann, K (2014) and Wang et al (2013) are duplicated. Also correct quotations.

We have corrected this.

Reviewer #2:

The manuscript entitled "The chromatin-associated protein PWWP INTERACTOR OF POLYCOMBS1 (PWO1) regulates nuclear morphology in Arabidopsis" submitted by Mikulski et al. describes the function of PWO1, a recently published PcG protein, and its relationship with the CRWN1 protein expected to be a major component of the plant lamin-like structure. The authors provide evidence that CRWN1 and PWO1 act in a common pathway and regulate a common set of genes using genetics and molecular approaches. I have no doubt that the authors bring a major contribution to the field. I however think that the manuscript should be revised before publication.

A first general comment is about the use of the term "nuclear lamina" for describing the structure associate to the CRWN proteins. Although it is very tempting to conclude that CRWNs are functional homologs of lamins, to my knowledge, there is no clear proof as we don't know if CRWNs form complexes, intermediate filaments or polymers and if they can interact with chromatin as described for LADs in animals. I would suggest using "lamin-like structure" instead of "nuclear lamina" (l113, l228...)

We agree with the reviewers that it is better to use lamin-like structure. We have corrected this throughout the text.

The second is the need to better clarify the concepts of facultative vs constitutive heterochromatin and LADs and their relationship with the PcG proteins. Constitutive heterochromatin and constitutive LADs are repressed during all the interphase while facultative heterochromatin and LADs can be active or repressed at the transcriptional level during the interphase. LADs are enriched in marks also found in heterochromatin (H3K9me2 and me3) but also in facultative heterochromatin (H3K27me3). What is the real connection between LADs and PcG proteins? Are there any references showing that LADs are affected in PcG mutants that the authors can cite in the introduction? Instead, can they clearly state that there is so far no clear relationship between LADs and PcG proteins?

This is a fair and important point. The connection between LADs (laminB1) and PcG/H3K27me3 is mostly focused on a (partial) overlap between H3K27me3 and laminB1 mapping (PMID: 18463634), whereas a more recent (and technically more advanced) study (PMID: 25825760) rather showed a correlation of H3K27me3 and iLADs. However, there is likely a distinction between different lamins as lamin A/C interact with PcG proteins and have an impact on nuclear interior PcG distribution (PMID: 26553927). Obviously, this are all data from mammals and the situation in plants may be different. To our knowledge, there is no report showing that lack of PcG affects LADs.

We have addressed this now more in the intro and discussion.

The third one is that the figures and supplementary figures have to be checked carefully.

Please see below for detailed replies.

More specific comments and suggestions:

Abstract

l32: I would suggest distinguishing between constitutive and facultative heterochromatin.

l34: "atypical": in which way the lamin-like structure of plants is atypical?

This has been modified.

Introduction

As a summary, we have addressed all the comments raised by the reviewer for the introduction. We believe that the intro now better introduces into the paper, particularly regarding plant nuclear-lamina like structures. We omitted an extensive review of lamin-like structures to keep the intro relatively short.

l55-l65: I think the authors should clearly distinguish the knowledge in animals vs plants, or they should focused only on plants. It could have been interesting to provide details of our current knowledge about the lamin-like structure in plants (Fiserova 2009, 2010), the discovery of NMCPs (Masuda 1997, Ciska 2013...). I would also suggest introducing the work of Sakamoto and Takagi 2013, who extracted proteins expected to be components of the lamin-like structure, as this reference set of proteins is extensively used in this publication. The reader should know more about this "crude nuclear lamina fraction".

This has been addressed, but only in the lower part of the intro, from lines 104 on.

l53: early cytological study "in plants" showed... chromocenters are also detected in a few other organisms, including mouse.

This has been addressed.

l74: can the authors distinguish facultative & constitutive heterochromatin (see also my comment in l32 in the abstract)?

This has been addressed.

l76-78: Can the author clarify the possible link between LADs and PcG regarding the enrichment of some fLADs in H3K27me3 marks?

We introduce cLADs, fLADs and iLADS now and cite relevant literature, we also mention the interaction of PcG proteins with lamin A/C, which is occurring in the nuclear interior. We believe that there is no clear connection between fLADs and H3K27me3 while this is tempting to speculate.

l87: Please revise this statement. Bi et al 2017 have used NUP1-GFP to immunoprecipitate chromatin located next to the nuclear periphery (indeed here next to the NPC). They neither used CRWN (the expected functional homolog of Lamin) nor the DamID technique (but instead the new RE-ChIP technique). It is therefore difficult to determine if they isolated LADs.

If the authors believed that the identified sequences are indeed LADs then it is tempting to suggest defining the overlap between H3K27me3 target genes / DEGs found in pwo1 and/or crwn1/2 and the regions identified in Bi et al.

We describe this now more clearly. We also performed the analyses but identified little overlap which may be explained by the fact that NUP1 is only associating with NPCs and not LADs.

l97-98: "a range" suggests that a large set of interactors have been already discovered. I suggest indicating clearly that CRWN1 has been shown to interact with SUN1 by FRET (Graumann 2014) and CRWN1 / KAKU4 by GFP-trap and Y2H and CRW4 / KAKU4 by GFP-trap (Goto et al 2014).

This has been addressed.

l99: facultative and constitutive heterochromatin were not introduced to the reader. Please try to introduce this concept earlier in the introduction.

This has been addressed.

RESULTS section

l133: The authors should identify the interacting domain between CLF and PWO1. A deletion analysis (removing the interacting domain) could be used to demonstrate that CLF/PWO1 interaction is required for co-localisation of both proteins in the same nuclear bodies.

We are not sure whether this dataset would really add to the manuscript. Nevertheless, we started analyses of the relevant constructs (based on old yeast two-hybrid analyses that the part of CLF that interacts with PWO1 is in the CLF-CXC or CLF-PRE-CXC). The experiments are not yet finished due to difficulties with recovery of clones and yeast two-hybrid analyses, but we believe the data would not add much to the current manuscript.

We have previously done deletion analyses of PWO1 (Fig. 6, Hohenstatt et al., PMID 29330200) and showed that a disruption of the N-terminal PWWP-domain results in loss of nuclear bodies. CLF on its own does not localize to bodies and/or to the nuclear periphery (Fig. 2 of Hohenstatt et al.). So it would be indeed interesting to remove the domains in CLF and/or PWO1, but would this tell much about the interaction of PWO1 with nuclear lamina-like components which is the focus of this paper?

Overall, we believe that the experiment suggested is very interesting, but would not strongly enhance the main message of the paper, but result in a significant delay of a potential publication of the paper.

l142: Figure 1: revise the legend as B) and D) have been swapped. Panel A should also show i35S::CLF-GFP and i35S::PWO1-mcherry alone. Revised "microns" to "µm" as for the other figures. The legend of Fig1B is not clear. I would suggest speckles (spec) vs non-speckles (non-spec). B) Speckle's amount: indicate the number of nuclei used in this experiment. The Gaussian blur is maybe not required in the legend.

We have modified the legends. The figure is somewhat a deeper analysis of the interaction of CLF-PWO1 analysed in Hohenstatt et al. (2018) (PMID 29330200), here focusing on the speckles and the subnuclear localization of the speckles. There, we show expression of the individual constructs and we think this is properly cited and referred to in the text (see first few sentences of results). We have modified the legend of 1B to make it more clear. For the speckles´ amount the number of nuclei is indicated (in total 13 nuclei), see x axis.

l151: Figure 2: the left-hand panel (zoomed-in image) at the top does not bring much as the image resolution is not sufficient. The nuclear speckles look very diffused, and it is therefore rather difficult to call them "foci". Would it be possible to get an image of better resolution? Please include a DAPI staining as a control (panel A, B and C) and

comment on the distribution of the speckles in respect to the chromocenters. Indicate the nucleolus (no) in the 2B panel.

We have used a super-resolution technique (Zeiss LSM880 with Airyscan) that improved the image resolution (see figure 2 and methods), but could not clearly resolve the issue raised by the reviewer, Still, root meristematic nuclei show a more focal-like PWO1 pattern than nuclei of differentiated cells, but it cannot be excluded that foci are connected. There is no clear co-localization with the nuclear lamina of PWO1-GFP. As PWO1-GFP shows only weak GFP signal (but complements the phenotypes) other methods such as SIM were not successful and STED would likely not be feasible with this line. Overall, we believe that the new analyses have clearly improved the resolution of the images.

DAPI staining interferes with the channel to detect GFP, therefore a co-staining is problematic. In the previous paper (Hohenstatt et al. (2018) (PMID 29330200)) we have performed immunostaining with this line (PWO1-GFP) and co-staining with DAPI (Figure 2c) which showed a foci-like pattern and more importantly, exclusion of PWO1 from chromocenters.

l169: I would suggest including the complete list of the GFP-trap/MS screen as a new supplementary table. This would be a valuable resource for the plant community. As an illustration of the value of this list, the authors used the list from crude laminar extract published by Sakamoto and Takagi 2013.

We now provide the full list in the supplementary data.

l191: is there any components of the PcG machinery in the crude nuclear lamina fraction?

Yes, MSI1 is a PRC2 member, identified in the PWO1 IP and in the crude nuclear lamina fraction (see text, line 215). MSI4 is also found the in the crude nuclear lamina fraction, but not in the PWO1 IP, however, we found that PWO1 interacts with MSI4 in yeast two hybrid (not shown).

L195: Table S1 present overlapping proteins with the crude lamina extract. This table should be sorted according to either the pValue or the log2FC to indicate the most significant hits at the top of the Table. While ribosomal proteins were removed according to the Legend of Table S1, 9 accessions numbers correspond to ribosomal proteins. Finally, the authors focused on CRWN1 following the GFP-trap/MS screen while many genes coding for histones (8 accession numbers) are also found in the screen. Three of them are H2A.W histone variants, suggesting a possible link with pericentromeric chromatin (see Yelagandula et al 2014)?

We have re-sorted the table and removed all mitochondrial or chloroplastic proteins, but nuclear encoded ribosomal proteins were kept. We indicate in the text that histones were found (line 208) but did not want to start speculations (at least in the paper) about other interactors. We also now mark proteins that were identified in the PEAT complex (which also contains PWO1) (Tan et al. 2018, PMID 30104406; not published when initially submitted the paper) (see new table S1). In this paper, they report a function for this complex in TE repression, therefore a link to H2A.W may be interesting. PEAT complex components are exclusively in the non-NL fraction.

l200, l232, l379: Wang, Dittmer and Richards is cited twice. Please revise in the Reference section

This has been changed.

l203-204: did the author address the CRWN1 localization at different days after agroinfiltration? Recruitment at the nuclear periphery may be a slow process in tobacco as p35S was used (high expression of CRWN1 may result in nucleoplasmic localization).

This is a good point but we have not carefully addressed this. However, we used an inducible 35S to limit overexpression. The construct, however, seems functional as it leads to nuclear deformations. In principle, the analyses would need to be done in Arabidopsis with endogenous promotors. However, as we have several lines of evidence for the interaction (Y2H, co-IP (Ms/Ms) and FRET) we believe that this justifies our conclusion that PWO1 and CRWN1 interact.

l206 and Figure 4: The authors should check by western blot analysis if their full-length proteins are expressed in yeast. "-Trp -Leu -His" is a test medium with low stringency while the "-Trp -Leu -Ade -His" is most commonly used

as a test medium (see l435). This should be indicated in the M&M section to inform the readers that Y2H interaction between PWO1 and CRWN1 is rather weak.

We have not carried out the western blot analyses as the PWO1 construct was previously shown to be functional (Hohenstatt et al. (2018) (PMID 29330200)). As PWO1-frag3 and CRWN1-full length show an interaction they are likely to be sufficiently expressed. The part about low stringency was added to the methods.

l220: Is fragment 3 sufficient to form nuclear speckles or to interact with CLF (same experiments as in Figure 1 but with fragment 3 of PWO1)?

Fragment 3 is not found in speckles (see Fig. S2). This is not surprising as PWO1 lacking the N-term PWWP domain does not form speckles (Hohenstatt et al. (2018) (PMID 29330200))

l235-237: Maybe the author should recall here the results obtained by Dittmer et al 2007 for crwn1

We have added this to the text.

l243-244 and Figure 5: The authors should try to use an ACP analysis of the individuals to clearly show that *crwn1* and *crwn1 swn* mutants display a different phenotype than Col-0, *pwo1 crwn1*, *pwo1* and *swn* mutants in a single graph.

We are not sure what is meant here. If a Principle Component Analysis is suggested we believe that this is not very feasible as we just have two components. We carried out one-way ANOVA which shows that *crwn1* and *crwn1 swn* belong to the same group as *pwo1 crwn1* and *pwo1*. We hope that this answers the reviewer´s concern.

l259-269, Figure S5 and S6: this is a very interesting question. However, it requires images of higher resolution, as foci are diffused (see comment for Fig. 2). Furthermore, the loss of function of CRWN1 could partially be complemented by the other CRWN members. I think in the current form, it is difficult to say that PWOs and CRWNs do not depend on each other.

We agree with the reviewer and changed the title to "Loss of PWO1 and CRWN1 localization does not depend on each other does not drastically affect CRWN1 or PWO1 localization, respectively". As this is very interesting it will require a much more detailed analysis with SRM techniques with extensive image analyses. In addition, there are factors such as redundancy etc.

l270: I think it would have been better to use the *crwn1* transcriptome instead of *crwn1 crwn2*. 1) CRWN2 has not been studied in the previous section of the paper and was not shown to interact with PWO1 (unless CRWN2 is among the 99 proteins list sorted from MS sequencing?). 2) *crwn1 crwn2* double mutant has a very strong effect both on nuclear morphology and chromocenter organization (fused chromocenters). The authors should explain why *crwn1 crwn2* was chosen instead of *crwn1*.

We initially chose the stronger *crwn1 crwn2* mutant to uncover a stronger effect on the transcriptome and perhaps observe a stronger overlap of genes mis-expressed in *crwn1 crwn2* and H3K27me3 target genes (than for *crwn1* singles). It probably would have been better to analyse *crwn1* single mutants in addition. Nevertheless, while we would expect fewer genes to be mis-regulated in *crwn1* mutants we believe it is unlikely that the level of significance between *pwo1* and *crwn1/2* is a mere result of using the double mutant.

l324: missing space

Changed.

l326: Although I agree with the fact that PWO1 regulates only a subset of PRC2 targets, a 3D image analysis is required here to define the exact location of H3K27me3 marks in respect to the nuclear periphery.

This is true although the main fraction of H3K27me3 is not at the periphery. It is an interesting possibility that some parts of H3K27me3 are at the periphery. We added "predominantly" to the H3K27me3 localization.

l341-343: this can be extended to the CRWN family which also display redundant functions among its members

We have added two sentences on the PEAT complex whose knock down is very similar to *pwo1/2/3* triple mutants.

l396-397: Please explain the use of the 4 replicates

As sometimes one of the replicates is not usable (due to insufficient amount of protein etc) 4 replicates are generated to permit the loss of one of the replicates. As all 4 replicates were ok, we included them all which enhances statistical analyses.

l398 and 399: Kaufmann 2010

We think the current reference is the correct one for the IP-Ms/Ms

l418-421: the method/plugin/software used to define distances is not described.

We added a sentence: "The distance was measured using Fiji/ImageJ software."

l481-482 and 486: can you reformulate to describe first Cufflinks and then EdgeR analysis?

We are not sure how to modify as we describe cufflinks first and then EdgeR: " Alignment files were processed in two different pipelines: 1) Cufflinks (Tuxedo protocol, (Trapnell et al., 2013)) and 2) EdgeR (Robinson et al., 2010). 1) FPKM values from mapped reads were calculated in Cufflinks v2.2.1 with enabled: reference annotation (-g), multi read correction (-u), fragment bias correction (-b) and minimal intron length set to 20 bp. Cufflinks output was used to create common transcript reference file in Cuffmerge v2.2.1.0 and perform DEG analysis in Cuffdiff v2.2.1 with multi read correction and fragment bias correction. 2) Mapped reads were transformed into counts using HTSeq v0.6.1 and used as input for edgeR v3.3. Features with less than 1 count per mln were discarded. For remaining features differential expression was computed with adjusted p-values <0.05. DEG analysis in either of pipelines concerned comparison between wildtype and mutant (pwo1 or crwn1/2) samples."

l488: « 1 count per mlm » please define mln

We added "mln reads"

TPC2018-00663-RAR1 2nd Editorial decision –*revision requested* **Jan. 22, 2019**

We have received reviews of your revised manuscript entitled "The chromatin-associated protein PWO1 interacts with plant nuclear lamin-like components to regulate nuclear size." Thank you for submitting your best work to *The Plant Cell*. The editorial board agrees that the work you describe is substantive, falls within the scope of the journal, and may become acceptable for publication pending revision, and potential re-review.

[Editor and Reviewer comments shown below along with author responses]

TPC2018-00663-RAR2 2nd Revision received **Feb. 26, 2019**

Reviewer comments and **author responses**:

Editor (from decision letter):

General: We ask you to pay attention to the following points in preparing your revision. As you will see from the detailed comments of two of the previous reviewers, they appreciate the improvements in the revised version and would like to see the work published. However, they both note the lack of methodological details in the manuscript: please add the requested information and include the minor corrections as suggested. More importantly, both reviewers see a problem in the interpretation of the high-resolution images of PWO1 localization if expressed from its own promoter in Arabidopsis. The editors agree with the reviewers that there is a discrepancy between these images and the overexpression data from Fig 1. in this manuscript and in Hohenstatt et al. 2018. We recommend modifying the interpretation, replacing the terms "foci/speckles" in case of the Arabidopsis data. This does not need new data, just a revision in the text throughout.

We thank the reviewers and editors for their positive evaluation of our manuscript. We have been careful in the interpretation of the subnuclear localization of PWO1 and it is not possible to say yet whether we do see speckles, foci, network etc, so we have chosen to call regions of higher PWO1 protein intensity "structures" or "subnuclear

structures" or "non-uniform localization" throughout the text. There is indeed a discrepancy between *Arabidopsis* and *N. benthamiana* but the two systems are only moderately comparable due to different genome size and organization and expression systems. The data between Hohenstatt et al. 2018 and this manuscript is consistent, so similar localization in *N. benthamiana* and similar localization in *Arabidopsis* (in Hohenstatt et al. 2018 immunostaining, here confocal microscopy of GFP tagged variants). We hope that the manuscript is now acceptable.

Reviewer #1:

As stated in the first revision the manuscript provides novel and very interesting information about the links between PWO1 and CRWN1 and has been improved with the revision. Nevertheless, the accurate localization of PWO1 in A. thaliana still not resolved with the application of the super resolution technique with Airyscan that shows a distribution of the protein mostly in an intricate nuclear network rather than in discrete foci, especially in the epidermis and cortex elongation zones (see Fig 2).

We have changed this in the text (see above) and discuss the different localization with more detail in the discussion.

Also, the authors should explain in the materials and methods section the conditions of sample preparation and processing in the different species and tissues to allow a better interpretation of the images.

This has been added.

Minor comments:

Line 97 lamin-like proteins

Changed.

Line 100 lamin-like genes

Changed.

Line 112 other plant

Changed.

Lines 335-336 Foci are not clearly observed in Arabidopsis, please change

Changed to: PWO1 in Arabidopsis show also a non-uniform localization in meristematic tissues; however the features and precise subnuclear position of observed structures require further exploration. Future works should also elucidate whether PWO1 structures in Arabidopsis are separate entities or form large interconnected subnuclear network

Line 377 see above

Changed to: Furthermore, our results on PWO1 subnuclear localization showed formation of predominantly peripheral speckles in *N. benthamiana* and non-uniform subnuclear localization in *Arabidopsis*.

Line 438 and following. Include the sample preparation procedures.

This has been added.

Line 456 and following. See above

This has been added.

Fig 2 Improve resolution and mark the putative foci in A and D

We have added tif files of the figure now with the highest resolution we have. Foci/structures are marked with arrows.

Reviewer #2:

The authors have carefully followed all the reviewers' recommendations and now provide a better version of the manuscript including a new title much more attractive.
The new fig.2 is of better quality.

Two minor remarks:
If possible, it would be nice to indicate in the methods section (l551) the total number of proteins (Fig.3), genes or transcripts (Fig. 6 and 7) used for the hyper-geometric tests as these numbers impact the final pValue.

We have added the relevant information.

I also noticed that a possible nucleolar localization of PWO1 speckles is mentioned in the discussion (l377) but was not illustrated or introduced in Fig. 1.

We have added a sentence in the results and methods, but removed "nucleolar" from discussion (+ two relevant references and sentence at l384), as the predominant localization in *N. benthamiana* was at the periphery and it's too speculative to make a link to the nucleolus which is also not the main point of the manuscript.

In the methods sections many "," instead of "." in concentrations.

This has been modified.

TPC2018-00663-RAR2 3rd Editorial decision – *acceptance pending* **Mar. 2, 2019**

We are pleased to inform you that your paper entitled "The chromatin-associated protein PWO1 interacts with plant nuclear lamin-like components to regulate nuclear size" has been accepted for publication in *The Plant Cell*, pending a final minor editorial review by journal staff. At this stage, your manuscript will be evaluated by a Science Editor with respect to scientific content presentation, compliance with journal policies, and presentation for a broad readership. *The Plant Cell* has appointed several Ph.D. Plant Scientists to serve as Science Editors in this capacity, and you will receive further information on this process very shortly.

We appreciate the answers to the reviewers' comments and the revisions of the text, including the modified description of the subcellular PWO1 distribution and consider the manuscript now suitable for publication in *Plant Cell*. However, it is unclear why you did not modify the abstract: "we demonstrate that PWO1 proteins forms foci located partially at the subnuclear periphery" is definitely not correct for the situation in Arabidopsis if PWO1 is expressed from the native promoter. Please revise the abstract accordingly during the final scientific editing stage. A science editor will be assigned to your manuscript and will contact you within the next week with an edited version; please wait to receive this correspondence and make this and any further changes to the edited version.

Final acceptance from Science Editor **Mar. 22, 2019**