Coordination of Meristem Doming and the Floral Transition by Late Termination, a Kelch Repeat Protein

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Plant Cell. Advance Publication April 7, 2017; doi: 10.1105/tpc.17.00030

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Review timeline:

TPC2017-00030-RA	Submission received:	Jan. 12, 2017
	1st Decision:	Mar. 7, 2017 *accept with minor revision*
TPC2017-00030-RAR1	1st Revision received:	Mar. 22, 2017
	2nd Decision:	Mar. 29, 2017 *acceptance pending, sent to science editor*
	Final acceptance:	April 5, 2017
	Advance publication:	April 7, 2017

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.)

TPC2017-00030-RA 1st Editorial decision – *accept with minor revision* Mar. 7, 2017

We have received reviews of your manuscript entitled "A kelch-containing regulatory hub orchestrates meristem doming with floral transition in tomato." On the basis of the advice received, the board of reviewing editors would like to accept your manuscript for publication in *The Plant Cell*. This acceptance is contingent on revision based on the comments of our reviewers.

All referees agreed that your findings were exciting and will significantly add to our knowledge of flowering time in species other than the Brassicaceae. However, Reviewers 1 and 2 noted some concerns in the interpretation of data regarding LTM and SP. Furthermore, Reviewer 2 noted that there was insufficient data that clearly demonstrate that LTM is a hub, that LTM acts as a repressor, nor that the physical interactions with TOPLESS and the other factors have any relevance to the doming or flowering time aspects of LTM function despite showing physical interaction and expression at the same time and the same place. We anticipate that these, and the other criticisms noted by all referees can be dealt with through rewriting without the performance of new experiments.

In particular, please consider the following:

*Please provide better images of LTM expression in vegetative apices relative to SP. The images provided in Figure 3E are of insufficient quality to draw the conclusions stated.

*Consider rephrasing your points in the results that imply that SP is the sole regulator of doming and flowering time. The data in Figure 7a do not support this conclusion.

*Reduce the sections and text describing the importance of the interactions between LTM, TPL and other factors, and reserve speculation about their influence on gene expression for the discussion (i.e not in the abstract). Furthermore, there are no data supporting LTM acting as a repressor - indeed the RNAseq profiling suggests it could act as an activator or a repressor.

*Please address the concerns of Reviewer 1 with respect to back-crossing.

*State appropriate caveats about the interpretation of meristem doming after flower transition in mutants as stated by Reviewer 2.

*Please further speculate in the discussion about the role of doming.

*Remove the word "hub" from the title.

* In Figure 5C please make it clear how many biological and technical replicates were used for the RT-PCR experiment.

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

[Reviewer comments shown below along with author responses]

TPC2017-00030-RAR1 1st Revision received March 22, 2017

Reviewer comments and **author responses**:

Dear Editors,

Thank you for the prompt and positive response to our work. We went over the comments, of the reviewers and yours, and tried to incorporate them in the most constructive way we could. We do feel the manuscript has been improved and some over reaching statements were toned down to better describe the available results.

Below we respond, point-by-point to the different concerns raised.

All referees agreed that your findings were exciting and will significantly add to our knowledge of flowering time in species other than the Brassicaceae. However, Reviewers 1 and 2 noted some concerns in the interpretation of data regarding LTM and SP. Furthermore, Reviewer 2 noted that there was insufficient data that clearly demonstrate that LTM is a hub, that LTM acts as a repressor, nor that the physical interactions with TOPLESS and the other factors have any relevance to the doming or flowering time aspects of LTM function despite showing physical interaction and expression at the same time and the same place. We anticipate that these, and the other criticisms noted by all referees can be dealt with through rewriting without the performance of new experiments.

Thanks. See below our specific, point by point responses.

*Please provide better images of LTM expression in vegetative apices relative to SP. The images provided in Figure 3E are of insufficient quality to draw the conclusions stated.

A new image now substitutes for the previous one. It clearly shows the uniform expression at the SAM. In addition, we provide a description of the SAM tissues taken for RNA seq and show that in the collected vegetative apices that are made mostly of the SAM, LTM expression level is as high as in meristems undergoing floral transition (now Figure 3 F).

*Consider rephrasing your points in the results that imply that SP is the sole regulator of doming and flowering time. The data in Figure 7a do not support this conclusion.

In the original submission we wrote (rows 342-3): "Taken together, both late-flowering and precocious PSM doming of *ltm* are caused by precocious SP expression in the vegetative apices."

We changed it now as follows: "Taken together, both late-flowering and precocious PSM doming of *ltm* are caused primarily, but not solely, by precocious SP expression in the vegetative apices."

*Reduce the sections and text describing the importance of the interactions between LTM, TPL and other factors, and reserve speculation about their influence on gene expression for the discussion (i.e not in the abstract). Furthermore, there are no data supporting LTM acting as a repressor - indeed the RNAseq profiling suggests it could act as an activator or a repressor.

We agree that some statements were taken beyond what the data show. We modified the abstract and the title, and also took away some of statements in the results as listed below.

Abstract (56-57) "LTM interacts with the TOPLESS (TPL) corepressor and with several transcription factors, providing specificity for its repressive functions.

Was changed to - "LTM interacts with the TOPLESS (TPL) corepressor and with several transcription factors, that may provide specificity for its functions.

Rows 278-280 – "To characterize processes and genes regulated by the putative LTM-TOPLESS-DNA-binding protein complex and which orchestrate doming with floral transition, global expression profiles of vegetative ltm apices were compared with those of same-age WT apices."

Was changed to - "To characterize processes and genes regulated by LTM containing complexes and which orchestrate doming with floral transition, global expression profiles of vegetative ltm apices were compared with those of same-age WT apices."

*Please address the concerns of Reviewer 1 with respect to back-crossing (145-150).

Thanks for this comment. We added a sentence to the methods saying – "To further understand the link between floral transition and SAM enlargement, we surveyed the apices of late-flowering mutants identified in a large mutant screen (Menda et al., 2004). These mutants were backcrossed 3-4 times to an isogenic indeterminate M82 (SP/SP) line before detailed characterization. Surprisingly, apices of one late-flowering mutant, which we termed late termination (ltm), showed precocious doming…."

*State appropriate caveats about the interpretation of meristem doming after flower transition in mutants as stated by Reviewer 2.

We believe that these comments are incorrect, at least for tomato. In our hands, WT meristems undergoing floral transition are indistinguishable from vegetative domed apices of the *fab* and *fas* mutants we looked at. Please compare Figure 1A (WT at TM) with Figures 2A and 6D (vegetative *fab* and *fas*).

*Please further speculate in the discussion about the role of doming.

A whole section in the discussion is dedicated to the role of doming. We now expanded that section to discuss the relations between the floral promoting and meristem inhibiting functions of florigen and its antagonist SP. We argue that these relations represent an emerging theme in plant hormone signaling – induction of antagonists by increased hormone signals. The whole modified paragraph is highlighted in the text.

*Remove the word "hub" from the title.

We have revised the title as follows: "Orchestration of meristem doming with floral transition by a kelch repeat protein."

* In Figure 5C please make it clear how many biological and technical replicates were used for the RT-PCR experiment.

OK. Biological replicates are in the legend and technical ones in the methods.

Reviewer #1:

A few minor comments. Recently a paper on flowering in tomato was published by the Lippman and Jimenez-Gomez groups (Soyk et al Nat Genet 49: 162). I wonder if this story is relevant for the present paper.

Soyk et al., described a different find of floral antagonist that is expressed in tissues where we could not detect LTM expression. We thus chose not to refer to this study.

Line 154- In Fig. 1c the WT data stop when the plants reach TM why not continue up to at least 11 days to show that the final meristem is much larger in the mutant.

Unlike with monopodial plants (Arabidopsis), in sympodial plants (tomato), once the meristem has reached floral transition, it turns into a flower. Thus, there is no point to continue measuring it.

Line 200. It might be useful to have a clear (summarizing) conclusion sentence on the previous negative data that there is no effect on WUS expression in the ltm mutant.

We write at the end of the paragraph – "Thus, distinct transcriptional signatures characterize the two mutants (Figure 2F), suggesting that their similar doming behavior is of different molecular origins.

One wonders what the role of Topless is in tomato. Is there a mutant and a mutant phenotype?

We apologize. Such lines are only being made. Their detailed characterization will require at least another growth cycle.

Similar questions might be asked about the other interactors and the relationship with JA signaling. The protein interactions are well described but not discussed very much in relation to the mode of action of LTM.

Unfortunately, speculations on the roles of the LTM interactors in meristem doming require genetic analyses that are beyond what we have to date.

Line 314 and 447. I assume that M82 is an *sp* mutant. How did the authors find the effect of *ltm* on dome size when the original mutant did not show this phenotype. By backcrossing with *SP*? If so should this not be mentioned?

Thanks for this correct comment. We took it (by mistake) for granted and now describe how it was isolated in the *sp* background and then backcrossed to *SP*$^{+}$ isogenic line (see comment above).

Reviewer #2:

Major points:

1. The authors spend a lot of the manuscript talking about doming and flowering time, but yet they don't really find anything concrete about how these two processes are related. There is a lot of speculation instead. The one gene they tested that partially rescued the late flowering time of *ltm*, *sp*, also rescued the precocious doming phenotype.

We did not say that doming and flowering time are related but that doming and flowering are. In fact, we show that the classical late flowering mutant *sft* has regular doming. In this work, doming and flowering time are linked, for the first time (as far as we know) and that linkage results by regulation of both process by the same gene, *SELF PRUNING*. We believe that this is a significant linkage.

2. Although *SP* is clearly up regulated in the young SAMs of *ltm* mutants, it is not clear from the *in situ* hybridizations that *LTM* is expressed in these SAMs in the wild type. *LTM* is highly expressed in the axillary meristems at this point, but so is *SP* according to the *in situs*. This should be explained or more convincing *in situs* of *LTM* should be shown.

To clarify this point we changed the image in Figure 3 and added more detailed description of the SAMs transcriptome. Please note that the expression of *SP* in the vegetative PSM samples is practically zero, but we did not include that result here due to the text flow. It is however part of the Heat Map in Figure 5. – see also above comments to the editor.

3-The identification of an EAR motif in LTM and its ability to interact with TPL is interesting, but all that is really shown is that they can interact, but not that this interaction has any functional relevance in the plant. The speculation that TPL, LTM, LIA form a complex is just speculation and there are no data presented that this complex actually exists. The fact that all three of these genes are expressed in SAMs of tomato is promising, but not convincing. There also isn't really any data that says LTM acts as a repressor given slightly more genes go up in *ltm* apices than go down.

Thanks for these comments. We probably took our wishful thinking a step too far. We made now several changes to tune down the implications of these interactions. We look forward for future analyses of respective mutants and higher order complexes.

Minor comment: I would argue that the title needs to be changed. There really aren't any data presented that suggests that LTM acts as a hub, at least biochemically.

Done as requested.

Ultimately I believe there are some nice analyses on an interesting mutant here. However, I think the paper needs to be restructured to tell a more focused story based on what was found, instead of so much speculation.

Changes, clarifications and shortening of the mechanistic conjectures were made throughout the text as described above.

I would also argue that the lack of connection between the CLV/WUS pathway and LTM be taken out or the discussion reduced, as mutants that have domed SAMs from that pathway tend to look quite different than those that have undergone a floral transition.

Here we disagree. In tomato, the available mutants with vegetative domed SAMs (*fas* and *fab*) are indistinguishable from WT meristem undergoing doming and floral transition. The mutants are very different from WT afterwards, but cannot be distinguished if one is looking at the SAM only.

Reviewer #3:

I did manage to find a typo on page 193 - change preciouses to precocious.

Thanks for the kind words and the comment. We changed the typo.

TPC2017-00030-RAR1 2nd Editorial decision – *acceptance pending* **Mar. 29, 2017**

We are pleased to inform you that your paper entitled "Orchestration of meristem doming with floral transition by a kelch repeat protein." has been accepted for publication in *The Plant Cell*, pending a final minor editorial review by journal staff.

Final acceptance from Science Editor **April 5, 2017**