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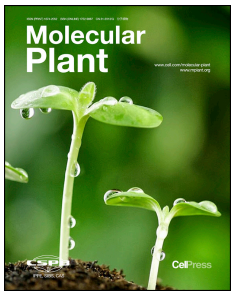

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TCP14 and TCP15 mediate the promotion of seed germination by gibberellins in *Arabidopsis thaliana*

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Dear Editor,

Seed germination is a major developmental transition in a plant's life that involves the concerted action of several genetic and physiological pathways(Holdsworth et al., 2008), and mostly consists in the resumption of embryo growth after a long quiescence imposed during seed maturation. In mature seeds germination is repressed by abscisic acid (ABA), while favourable environmental conditions promote gibberellin (GA) biosynthesis and decrease ABA(Holdsworth et al., 2008). The increase in GA levels is essential for the rupture of testa and endosperm(Lee et al., 2002). The activation of cell division in the embryois an integral part of germination that precedes the protrusion of the root through the seed coat. In *Arabidopsis*, this activation has been linked to significant early changes in the expression of cell-cycle elements (Masubelele et al., 2005). In the root apical meristem (RAM) GAs regulate cell divisions (Achard et al., 2009; Úbeda-Tomás et al., 2009). Similarly the transcription factors (TFs) TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR14 (TCP14) and TCP15 have been proposed to regulate cell proliferation(Kieffer et al., 2011). Moreover, the activity of TCP14 is necessary to undergo seed germination and remarkably, seeds lacking this activity are hypersensitive to the negative effects on germination of the GA biosynthesis inhibitor paclobutrazol, suggesting a functional relationship between this TF and GAs(Tatematsu et al., 2008). Taken these observations into account, we have hypothesized that TCP14 and TCP15 mediate the GA-dependent activation of the cell cycle during germination.

First, we tested whether TCP15 activity is also necessary for germination. *TCP15* disruption in the *tcp15* mutant, like *tcp14*, delayed seed germination (Figure 1A). In

agreement with the requirementfor*TCP15*, it is expressed in developing embryos and in seeds during germination (Figure S1), as already shownfor *TCP14*(Tatematsu et al., 2008). *TCP14* and *TCP15* genes are closely related phylogenetically, suggesting that their activities might be redundant to some extent(Danisman et al., 2013). We thereforemeasured the germination ability of *tcp14 tcp15* mutant seeds. The *tcp14 tcp15*seeds showed a more severe reduction in the germination capability than any of the single mutantsand,strikingly,neither GA application nor exposure to cold,that enhances GA accumulation,were able to efficiently overcome this defect, while a full recovery was obtained in the case of single mutants (Figure 1A). These dataindicate that both TCP14 and TCP15 act downstream of the GA and the stratification pathways that promote germination.

GAs regulate cell elongation and cell division by promoting the degradation of the DELLA proteins, which are the negative regulators in the GA signalling pathway(Locascio et al., 2013). Optimal germination requires the induction of GA biosynthesis to counteract the repressive effect imposed by DELLAs. Given that TCP14 and TCP15 are necessary for root emergence (Figure 1A), we hypothesized that DELLA activity might prevent germination, at least in part, by repressing these two TCPs in dormant embryos. However,the expression of *TCP14* and *TCP15* was not altered in response to the conditional accumulation of the dominant version of the DELLA protein GAI, gai-1, in the *HS::gai-1*transgenic line(Alabadí et al., 2008), suggesting that if DELLAs regulate TCP activity, it should be at the post-transcriptional level(Figure S2). DELLAs modulate multiple signalling pathways through physical interaction with manyTFs(Locascio et al., 2013).Therefore, we tested if this would be the case for TCP14 and TCP15. Yeast two-hybrid assays demonstrated that TCP15 and TCP14areable to interact with GAI and withRGL2 (Figures 1B and S3A), the twoDELLA proteins with a more relevant role in theregulation of germination(Lee et

al., 2002). Similarly, *myc*-GAI was efficiently pulled down in coimmunoprecipitation assays withanti-HA antibodies from leaf extracts that co-expressed HA-TCP14 (Figure 1C). Bimolecular Fluorescence Complementation assays in *Nicotianabenthamiana* leaves further confirmed the interaction between the TCPs and GAI and RGL2 (Figures 1D and S3B).

Next, we designed an *in vivo* transcriptional assay in leaves of *N. benthamiana* to test our hypothesis that DELLA proteins inactivate the TCPs upon interaction. For this aim, we prepared a synthetic promoter consisting of six repeated copies of the TCP class I consensus binding site (GTGGGCCCAC) separated by a 6 nucleotide spacer (AAAAAA), and placed upstream of a minimal *35S* promoter and the viral translational enhancer Ωto control the expression of the reporter gene *LUCIFERASE* (*LUC*). Expression of a translational fusion of TCP14 with the transcriptional activator VP16 (3xHA-VP16-TCP14) caused an increase in the LUC activity (Figure 1E). Remarkably, the activation ability was largely reversed when GAI-TAP was co-expressed with 3xHA-VP16-TCP14, whereas GAI-TAP alone did not affect significantly the activity of the reporter (Figure 1E),suggesting that DELLAs inactivate the TCPs upon interaction. Interestingly, a recent report shows that DELLAs prevent the TCP's ability to bind DNA by physical interaction (Daviere et al., 2014).

It is well established that cell division occurring at embryonic root tips is important to promote germination(Masubelele et al., 2005).Kieffer and co-workers (2011) have shown that TCP14 and TCP15 jointly regulate plant stature by promoting cell proliferation in young internodes. Therefore we investigated whether these two TCPs are able to regulate cell division at the root apex of germinating embryos. The protodermain theRAM of the double *tcp14 tcp15*and the single *tcp15*mutants developed fewer cells than the wild type control, whereas the numberof cells in the *tcp14* RAMwas not significantly altered (Figure 1F). The role for TCP14 in this response, however, is

manifested by the greater impact that the double mutant has in this response compared to the single *tcp15*. Consistent with the negative effect that DELLAs exert on TCP's activity, GA addition greatlyrestored the cell number in the RAM of*tcp15*but not in that of *tcp14 tcp15*mutant embryos (Figure 1F).Interestingly, the module DELLAs/TCPs seems to exert its control specifically over cell proliferation but not over cell size (Figure 1F). To further support the role for TCP14 and TCP15 in controlling cell proliferation at the embryonic RAM, we assessed the activity of *CYCB1;1::GUS*, a well established marker for cells undergoing active divisions(Colon-Carmona et al., 1999), in the *tcp14 tcp15* mutant. The activity of a similar marker is regulated by TCP14(Tatematsu et al., 2008), and so is the expression of the *HISTONE H4* (Figure S4). Accordingly, *CYCB1;1::GUS* activity was strongly diminished in the double mutant compared to the wild type (Figure 1G) and, importantly, the ability of GAs to promote its activity was mostly suppressed in mutant embryos.

Next we asked whether TCP14 and TCP15 are involved also in controlling cell divisions in the RAM at later stages of development.We first tested this possibility by measuring root length in young seedlings, as this trait might be affected as a consequence of a defect in the cell division at the RAM. All mutants had a shorter root than the wild type, being the effect stronger in *tcp14 tcp15* seedlings (Figure S5A). Importantly, the positive effect of GA applicationon the final root length was seriously compromised in the double mutant, indicating that the activity of both TCPs is required for GAs to control this trait.In agreement with TCP14 and TCP15 controlling cell cycle at the RAM also in seedlings, *CYCB1;1-GUS* activity was diminished in the double mutant and was almost insensitive to treatments with GAs (Figure S5B).

In recent years several works have provided genetic support for the involvement of DELLAs' interactors in the control of seed germination (Park et al., 2013; Zhang et al., 2011). Moreover, control of DELLAs over cell cycle progression at the RAM has also

been reported (Achard et al., 2009; Úbeda-Tomás et al., 2009). Nonetheless, amolecular mechanism explaining the action of DELLAs on seed germination has not been proposed.In this work we provide evidences of such a mechanism, showing that the transcriptional regulators DELLAsmaintain the embryo in a quiescent state by restricting cell cycle progressionin the embryonic RAM through the inhibition of TCP14 and TCP15 activities upon interaction.Given the strong influence of environmental conditions on DELLA levels (Claeys et al., 2012), the DELLA-TCP module acts as a relay for environmental information into the cell cycle at the RAMto coordinate root emergence with other events during seed germination.

SUPPLEMENTARY DATA

Supplementary Data are availableat *Molecular Plant* online.

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FIGURE LEGENDS

(A) Germination assays showing that GAs (1μM GA_3) and stratification cannot fully overcome the delay imposed in the double mutant *tcp14 tcp15*. Freshly harvested seeds were used in these assays.

(B) Yeast two-hybrid assays demonstrating that TCP14 interacts physically with the DELLA proteins GAI and RGL2.

(C) Co-immunoprecipitation performed in leaves of *N. benthamiana* showing the interaction between *myc*-GAI and HA-TCP14. Immunoprecipitation was performed with anti-HA antibodies.

(D) Bimolecular fluorescence complementation assays showing the interaction between GAI and TCP14 in nuclei of epidermal cells of *N. benthamiana* leaves.

(E)GAI inhibits TCP14. Cells of *Agrobacterium*harboring the reporter construct were infiltrated in leaves of *N. benthamiana* alone or together with cells harboring effector constructs (TCP14, TCP14/GAI, or GAI). Firefly LUC activity was normalized to *Renilla* LUC. Values were normalized with respect to the ratio obtained for the reporter construct alone. Three biological repeats were performed, and error bars represent SEM.

(F) Graphs showing the number of cells at the protoderma and cortex of the RAMat embryonic stage in the wild type and mutants, as well as the effect of GA-treatment.

(G)TCP14 and TCP15 can regulate cell cycle at the RAM. *CYCB1;1::GUS* expression in wild type and double mutant embryos with or without GA treatment.