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Identification of gene functions associated to active and dormant buds in *Arabidopsis*

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In *Arabidopsis*, axillary buds may become dormant in response to a far-red rich light or to increased apical dominance. *BRANCHED1* (*BRC1*) is required for this response. Transcriptional profiling studies of wild-type and *brc1* mutant buds allowed the identification of sets of *BRC1*-dependent genes including a group of ABA-related genes upregulated in dormant buds. By using *BRC1* inducible lines we demonstrate that 2 of these ABA response factors, *ABF3* and *ABI5,* are positively regulated in axillary buds by *BRC1* after induction. To get further insight into the genetic control of the growth-to-dormancy transition in buds we have also compared this transcriptomic data with 2 additional "active vs dormant bud" transcriptomic data sets and found "core" co-regulated gene networks tightly associated to each condition.

Axillary buds are small structures localized at the base of leaves. They are branch primordia that contain, preformed, all the elements of an adult branch: a shoot apical meristem, several leaf primordia, a compressed shoot and, sometimes, inflorescence and flower meristems. Axillary buds may continue to grow until they elongate to give a branch or they may become quiescent until endogenous and environmental conditions are optimal for their development. One environmental signal controlling bud activity is a change in light quality, in particular, in the red (R) to far-red (FR) light ratio (R:FR). During photosynthesis, leaves absorb R and reflect FR, therefore plants interpret a decrease in R:FR as a signal of impending shading by neighboring vegetation. In low R:FR, plants trigger a group of developmental responses collectively known as *shade avoidance syndrome* (SAS) one of which is the promotion of axillary bud arrest and suppression of lateral shoot outgrowth.

In *Arabidopsis*, this response requires the *BRANCHED1 (BRC1)* gene function. *BRC1*, expressed in axillary buds and encoding a class II TCP transcription factor,[1-3] delays bud growth and development, and promotes bud dormancy. In a recent work, published in Plant Cell[4] we showed that under a FR-rich light, axillary buds have 2 to 3 times more *BRC1* mRNA levels than in high R:FR. Moreover, *brc1* mutants can develop many branches in the shade, unlike wild-type plants. To further investigate *BRC1* function in the SAS, we looked for genes downstream of *BRC1* in low R:FR. For that, we treated *Arabidopsis* wild-type and *brc1* plants (whose axillary buds were beginning to develop) with 8 h of low R:FR and studied gene expression in axillary buds and subtending tissue (**Fig. 1A**). In wild type, bud dormancy markers were upregulated after the treatment whereas their levels were unchanged in *brc1* mutants. This confirmed that wild-type but not *brc1* buds were becoming dormant after the treatment. We then expanded this analysis by performing microarray hybridizations with the same mRNAs. We hypothesized that genes whose expression changed in low R:FR in wild type but not in *brc1* required *BRC1* function for their regulation and termed them *BRC1*-dependent genes. We identified 2 *BRC1*-dependent networks of cell cycle- and ribosome-related genes significantly downregulated in wild-type.[4] Genes in these networks have promoters enriched in TCP binding sites, indicating that they could be directly controlled by TCP factors. A tight relationship between *BRC1* function and the regulation of some of the cell cycle-related genes was further supported by their downregulation in estradiol-inducible *BRC1* seedlings grown in high R:FR.

In addition, a significant proportion of ABA-responding genes[5] were induced in low R:FR in wild type, indicating high ABA signaling. In contrast, ABA response was significantly reduced in *brc1* mutants. Global analysis of upregulated gene promoters showed a significant overrepresentation of the aCACGTGt motif, which contains the ABA Responsive Element (ABRE, ACGT).[6] All these results agreed with an activation of ABA signaling in buds entering dormancy in low R:FR, and with a role of *BRC1* in maintaining this response. ABA has been classically associated to bud dormancy in many species, however in *Arabidopsis* this association remained elusive. In a recent study,[7] Reddy et al. quantified ABA levels in axillary buds with different growth capabilities (due either to their node position or to exposure to different light conditions) and confirmed that ABA abundance directly correlated with the degree of bud dormancy also in *Arabidopsis*. Moreover, they showed that ABA biosynthesis mutants *nced3–2* and *aba2–1* had enhanced branching in low R:FR. Finally, transcriptomic analyses of inactive vs active axillary buds were also consistent with a high ABA

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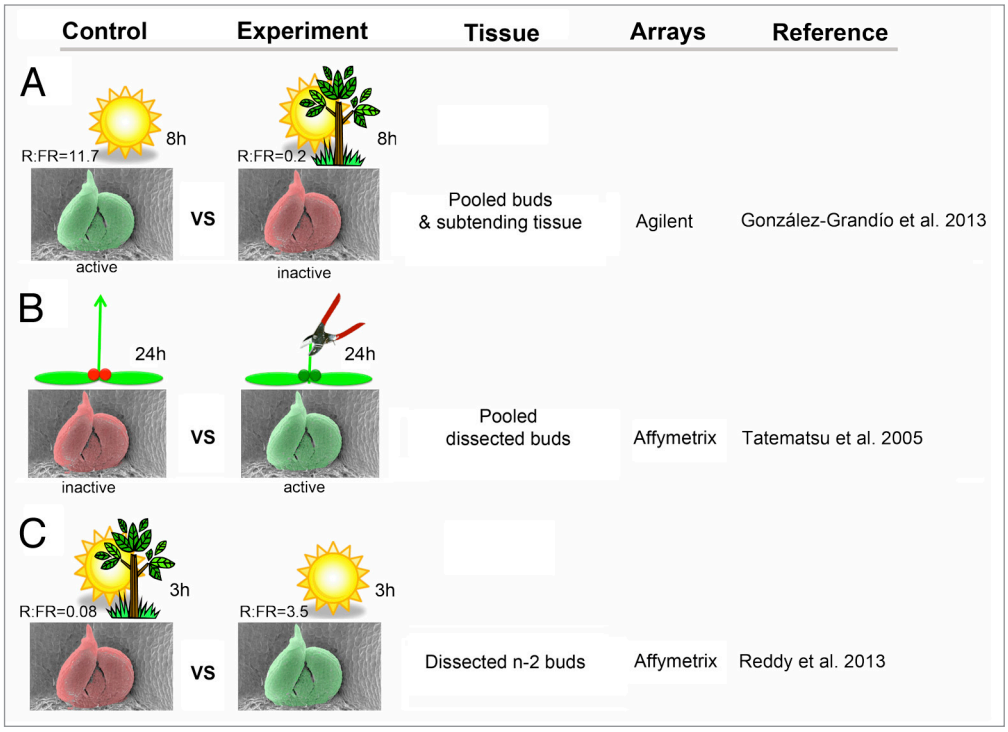

Figure 1. Transcriptomic profiling experiments comparing active vs dormant buds in *Arabidopsis*. (**A**) axillary bud dormancy was induced by an 8-hour treatment of low R:FR = 0.2. (**B**) bud activation was induced by decapitation of the main apex. Buds of intact or decapitated plants were pool-collected and transcriptional profiles compared after 24 h. (**C**) bud activation was induced by exposing plants grown in shade (R:FR = 0.08) to high R:FR = 3.5 for 3 hours. Bud n-2 (n being the uppermost rosette bud) was analyzed. Red, dormant buds. Green, active buds.

signaling in inactive buds. All these results support our findings. One possibility to explain the observed relationship between *BRC1* and ABA signaling could be that *BRC1* promotes the expression of key ABA transcription factors such as *ABF3* and *ABI5*. These genes are *BRC1*-dependent and contain TCP binding sites in their promoters. To test this, we have studied their transcriptional response in *BRC1* estradiol-inducible lines. Our results confirm that both genes are quickly upregulated in axillary buds by the sole induction of *BRC1,* even in high R:FR (**Fig. 2**). These results indicate that, although the molecular mechanisms are still unclear, ABA signaling probably plays an important role in the control of axillary bud arrest in *Arabidopsis* and that *BRC1,* required to maintain ABA signaling, could be regulating the transcription of essential ABA-related factors.

To identify general gene functions controlling bud activity in *Arabidopsis* irrespective of the inducing signals involved, we compared our transcriptomic analyses with 2 additional ones in which mRNA of active vs inactive buds were analyzed. In these studies bud activation was triggered either by decapitation of the main apex,[8] (**Fig. 1B**) or by exposure of plants grown in low R:FR to a short period of high R:FR[7] (**Fig. 1C**). Thus, the 3 experiments used different treatments, bud material as mRNA source, microarrays platforms (Affymetrix or Agilent), and sampling time points (**Fig. 1**). Genes changing similarly in all active or dormant bud samples should be tightly associated to bud growth activity. As the experiments displayed different transcriptomic profiles, we selected the 1000 genes most significantly (lowest FDR) up- and downregulated for each data set, regardless their fold change (provided it was >1.1 or <-1.1, **Table S1**) and we looked for common elements using Venny (http://bioinfogp.cnb.csic.es/tools/venny/). Twenty-five genes (termed *bud activation genes*) were upregulated in all 3 *active bud* samples and downregulated in all 3 *dormant bud* samples. Likewise, 78 genes (*bud dormancy genes*) were up and downregulated in dormant and active buds, respectively, in the 3 data sets.

Bud activation genes fell in 3 groups of co-regulated genes (ATTED-II[9]) related to DNA replication, S phase and mitosis (7 genes), flavonoid synthesis (5 genes), and cytokinin signaling (11 genes) (**Figure S1**). The cytokinin-related network comprised genes encoding proteins involved in cell wall synthesis, sucrose, fatty acid, and phenylpropanoid metabolism, several of them targeted to plastids, mitochondria, and vacuole (**Table S1**). Two additional identified genes were related to ribosome biogenesis (At5g61220) and RNA processing (At5g63120), respectively.

Bud dormancy genes fell into 4 groups of co-regulated genes. The largest group comprised 26 genes related to ABA, including the ABA synthesis gene *NCED3*, *HIS1–3*, and the transcription factors *ABI1*, *ABF3*, *AFP1*, *AFP3*, *NAC019*, *NAC092*, and *NAP*, further confirming the implication of ABA in bud dormancy (**Figure S2**). A group of 10 co-regulated genes included ethylene and auxin-related genes such as *WES1*, involved in auxin synthesis, ethylene receptor *ERS2*, the transcription factors *TEMPRANILLO*, *HAT4*, and *SHINE3* and the F-box *EBF2* (**Figure S3**). A group of 20 genes was highly upregulated in dark and night-extension treatments[10] (according to Genevestigator[11]) and strongly downregulated by glucose or sucrose treatments.[12,13] Members of this group were

related to sucrose metabolism and signaling (*COR414-TM1, AKINBETA1*), senescence (*SAG21*), protein degradation (RING and F box proteins), and abiotic stress (*DNAJ11, DNAJ20*) (**Figure S4**). Another group of 19 genes comprised proteins involved in autophagy (*NUDIX HYDROLASE HOMOLOG 15*), protein and aminoacid degradation (*BTB, FBP7, HGO*) vesicular transport (*MEMBRIN11*), and genes encoding proteins targeted to vacuoles and endoplasmic reticulum (**Figure S5**).

Genes identified as *bud dormancy* and *bud activation genes* constitute very early markers (they respond after 3 hours) of eco and paradormancy (they respond to changes in light quality and decapitation) in *Arabidopsis*. Moreover, these genes must have a sustained response as they are still differentially expressed 24 h after beginning of the treatment.[8] In addition, their regulation must be positively and negatively controlled as they display opposite behaviors during bud activation[7,8] and bud dormancy induction.[4] Some of them could play key roles in the coordinated regulation of gene expression leading to changes in bud growth status. The identification of these central factors will help us understand this process in *Arabidopsis*. Comparison of these profiles with those of more distantly related species in which bud responses have also been carefully analyzed (e.g., Vitis,[14] populus[15]) should help us find even more common themes in the control of bud dormancy and bud release.

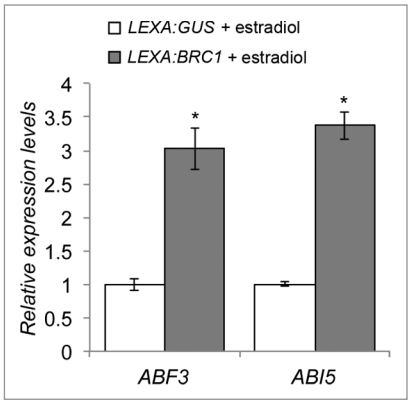

Figure 2. Response of ABA related factors to *BRC1* induction. mRNA levels of *ABF3* and *ABI5* were measured by qPCR as described in González-Grandío et al. (2013). Estradiol-inducible *BRC1* plants and estradiol-inducible *GUS* plants (control) were treated with 20 μM estradiol for 8 h. Error bars are SEM of 3 biological replicates of 8 plants each. Asterisks are significant differences (Student *t*-test, $P < 0.05$). Primers used were CCAAAGAGCG CCCTGGAT and TTTTCTCACT CGCCCAAACA T for *ABF3*, and CACCAGTTCA GGCAGGTGTT T and TGTCCACCCG CTCCAAA for *ABI5*. *SAND* was used as a reference gene.[16]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplementary Material

Supplementary material may be found here:
http://www.landesbioscience.com/journals/psb/article/27994

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