

Article Addendum

Role of TCP Gene *BRANCHED1* in the Control of Shoot Branching in Arabidopsis

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ABSTRACT

Branching patterns are major determinants of plant architecture. They depend both on leaf phyllotaxy (branch primordia are formed in the axils of leaves) and on the decision of buds to grow out to give a branch or to remain dormant. In Arabidopsis, several genes involved in the long-distance signalling of the control of branch outgrowth have been identified. However, the genes acting inside the buds to cause growth arrest remained unknown until now. In the February issue of *Plant Cell* we have described the function of *BRANCHED1* (*BRC1*), an Arabidopsis gene coding for a plant-specific transcription factor of the TCP family that is expressed in the buds and prevents their development. Loss of *BRC1* function leads to accelerated AM initiation, precocious progression of bud development and excess of shoot branching. *BRC1* transcription is affected by endogenous and environmental signals controlling branching and we have shown that *BRC1* function mediates the response to these stimuli. Therefore we have proposed that *BRC1* function represents the point at which signals controlling branching are integrated within axillary buds.

Plant branching patterns are mainly determined by an apparently simple decision: whether axillary buds formed at the base of leaves, grow out to give a branch or whether they remain small and dormant for long periods of time. This key decision determines the number of active axis of growth, leaves, flowers, fruits and seeds that the plant will produce. When growing shoots become damaged or senescent, plant survival depends on its capability to produce new shoots from axillary buds. On the other hand, adverse environmental conditions usually promote bud arrest. This prevents untimely branching which would compromise the fertility and viability of the plant. Therefore, axillary bud activity can be modulated by developmental and environmental stimuli perceived in different regions of the plant which, through long-distance signalling, are transduced into the bud to be translated into a decision of bud arrest or bud activation.

Bud arrest, or **bud dormancy**, is a reversible state that allows the plant to adapt to changing conditions. Depending on the factors promoting it, it has been termed **para-**, **eco-** or **endodormancy**.^{1,2} **Paradormancy** or **apical dominance**, is caused by an actively growing primary shoot apex^{3,4} and can be reversed by decapitation or pruning. **Ecodormancy** is a bud arrest imposed by limitations in environmental factors. **Endodormancy**, typical of woody plants, is a deep dormancy of the meristem caused by internal bud signals and usually requires a long exposure to chilling to be reversed.⁵ In the case of paradormancy, long-range signalling is mediated both by auxin, produced in the shoot apex and transported **basipetally**, and by a novel carotenoid-derived compound which modulates auxin transport, synthetised in the root and transported **acropetally**.^{3,6-8} On the other hand, cytokinin, a hormone produced in the root and stem, can enter the bud to promote bud outgrowth.

BRANCHED1 CONTROLS LATERAL SHOOT DEVELOPMENT IN ARABIDOPSIS

We used a molecular genetic approach to investigate the function of two Arabidopsis genes, *BRANCHED1* (*BRC1*) and *BRANCHED2* (*BRC2*), closely related to the maize, *teosinte branched1* (*tb1*) gene.⁹ Based on the known function of *tb1*, a key regulator of the apical dominance of maize, these genes were good candidates to control bud arrest in Arabidopsis. *BRC1* and *BRC2* belong to a small group of class II TCP transcription factors¹⁰ which includes *tb1*,⁹ the Antirrhinum gene *CYCLOIDEA*¹¹ and the Arabidopsis gene *TCPI*.¹²

A detailed phenotypic analysis of *brc1* and *brc2* mutants showed that, while *BRC2* has an almost irrelevant role during axillary bud development, wild-type *BRC1* delays axillary meristem initiation, axillary bud development and branch outgrowth.¹³

To investigate the genetic interactions between *BRC1* and other genes involved in axillary bud development, we studied *BRC1* mRNA levels in branching mutants and made double mutants with those and *brc1*. Our results indicate that *LATERAL SUPPRESSOR* and *INTERFASCICULAR FIBERLESS1/REVOLUTA*, two genes required very early during AM formation, are epistatic to *BRC1*. In addition, *BRC1* seems to be downstream of the *MAX* pathway. This genetic pathway, which includes the genes *MAX1*, *MAX2*, *MAX3* and *MAX4*, controls the synthesis and perception of a mobile carotenoid signal that promotes bud arrest.^{8,14-17} *MAX2/ORE9* is involved in the perception of the signal and seems to be required at the nodes of the plant, close to the site of action of *BRC1*.¹⁸ *brc1 max* double mutants are phenotypically similar to the mutant parents. Moreover, in *max* mutants, *BRC1* mRNA levels are greatly reduced. *MAX2/ORE9* codes for an F-box protein likely to be involved in ubiquitin-related protein degradation. A possible scenario is that *MAX2* could promote the degradation of a repressor of *BRC1* transcription so that, in *max2* mutants, the repressor would accumulate causing *BRC1* downregulation.

We have found that *BRC1* is both, quickly down-regulated after decapitation (a stimulus that promotes branch outgrowth) and upregulated in high-density grown plants (a condition that promotes bud arrest). These results indicate that *BRC1* mRNA levels inversely correlate with bud activity. The additional observation that *brc1* mutants are partially insensitive to decapitation and planting density supports the view that *BRC1* mediates bud response to these signals.

BRC1 PROTEIN MAY ACT AS A TRANSCRIPTIONAL REGULATOR

BRC1 transcription is restricted to developing axillary buds suggesting that it acts locally, within bud cells to cause developmental arrest. How does the *BRC1* protein prevent bud growth? *BRC1* encodes a nuclear class II TCP protein likely to act as a transcriptional regulator.^{10,19-21} *BRC1* could, for instance, repress the transcription of genes involved in cell division. Interestingly, class I TCP genes, expressed in proliferating cells, seem to promote cell division and growth. This is supported by the gene targets proposed for some of them (i.e., PCNA, CYCLIN b, Pur- α , ribosomal proteins).²⁰⁻²³ As class II and class I proteins recognise overlapping DNA motifs, both types of proteins could bind to the same gene promoters causing opposite transcriptional responses. Alternatively, *BRC1* could form inactive heterodimers with class I proteins or their partners through interaction with the TCP domain.^{21,24,25}

EVOLUTION OF *TB1*-LIKE GENES IN ANGIOSPERMS

How does the function of *BRC1* relate to that of the monocot gene *tb1*? In monocots, a single type of *tb1/CYC*-like gene has been identified, while in dicots three types are present: *BRC1*-like (also called *CYC1*,²⁶ *BRC2*-like (*CYC3*) and *TCPI/CYC*-like (*CYC2*). It has been proposed that, at the base of eudicots, duplications of a single ancestral gene gave rise to these three types of genes.²⁶ Following duplications, the functions of the ancestral gene may have been unequally preserved in the three clades.^{27,28} This would explain why some functions during inflorescence and flower development observed in *tb1*⁹ seem to have been lost in *BRC1*, while *BRC2*-like and *TCPI/CYC*-like genes do not control shoot branching but are

expressed in flowers^{12,26} and, in some species have been shown to play key roles during flower development.^{11,29-31} It is possible that the ancestral *TB1*-like gene controlled the growth patterns of all axillary structures, both vegetative and reproductive and in dicots, after duplication, sub-functionalization led to the separation of vegetative and reproductive functions of these TCP genes.

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