Developmental Cell, Vol. 8, 443–449, March, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.devcel.2005.01.009

MAX1 Encodes a Cytochrome P450 Family Member that Acts Downstream of *MAX3/4* to Produce a Carotenoid-Derived Branch-Inhibiting Hormone

Jonathan Booker,[1,4] Tobias Sieberer,[1,4] Wendy Wright,[1,4] Lisa Williamson,[1] Barbara Willett,[1] Petra Stirnberg,[1] Colin Turnbull,[2] Murali Srinivasan,[3] Peter Goddard,[3] and Ottoline Leyser[1,*]

[1]Department of Biology
Area 11
University of York
York, YO10 5YW, United Kingdom

[2]Department of Agricultural Sciences
Imperial College at Wye
University of London
Wye, TN25 5AH, United Kingdom

[3]Yara International ASA
Bygdoy Alle 2
020, Oslo, Norway

Summary

The plant shoot body plan is highly variable, depending on the degree of branching. Characterization of the *max1–max4* mutants of *Arabidopsis* demonstrates that branching is regulated by at least one carotenoid-derived hormone. Here we show that all four *MAX* genes act in a single pathway, with MAX1, MAX3, and MAX4 acting in hormone synthesis, and MAX2 acting in perception. We propose that MAX1 acts on a mobile substrate, downstream of MAX3 and MAX4, which have immobile substrates. These roles for MAX3, MAX4, and MAX2 are consistent with their known molecular identities. We identify MAX1 as a member of the cytochrome P450 family with high similarity to mammalian Thromboxane A_2 synthase. This, with its expression pattern, supports its suggested role in the MAX pathway. Moreover, the proposed enzymatic series for MAX hormone synthesis resembles that of two already characterized signal biosynthetic pathways: prostaglandins in animals and oxilipins in plants.

Introduction

The basic body axes of plants are established during embryogenesis. The apical-basal axis is defined by the establishment of the shoot and root apical meristems at either end of the embryo. Postembryonically, stem cell populations in these meristems give rise to the entire shoot and root systems in a modular fashion. For example, the primary shoot is made up of units consisting of a stem segment, a leaf, and an axillary shoot meristem in the leaf axil (Leyser, 2003). The axillary meristem can activate to produce a new lateral axis of growth, and hence a side shoot or lateral flower. Thus, the final shoot body plan is highly variable, depending on the activity and fate of the axillary meristems.

Axillary meristem activity is influenced by a wide range of factors, including hormonal signals and environmental cues (Cline, 1991). Classically, hormonal control has been attributed principally to auxin and cytokinin. Auxin, derived from the primary shoot apex, is pumped down the plant in the polar transport stream and inhibits the outgrowth of axillary buds. Because this auxin does not enter the bud, its inhibitory effects must be indirect (Morris, 1977; Booker et al., 2003). Cytokinin synthesized in the roots is transported up the plant in the transpiration stream in the xylem and can act directly in buds to promote their outgrowth (Cline, 1991). Basally supplied cytokinin can overcome the inhibitory effects of apically applied auxin in isolated *Arabidopsis* nodes (Chatfield et al., 2000). The inhibitory effect of auxin is likely partially mediated by its ability to reduce both cytokinin export from roots and cytokinin synthesis locally at the node (Bangerth, 1994; Nordstrom et al., 2004).

As well as these classical hormones, recent results show an additional carotenoid-derived hormone is involved. Mutations at the *MAX1–MAX4*, *RMS1–RMS5*, and *DAD1–DAD3* loci of *Arabidopsis*, pea, and petunia, respectively, confer increased shoot branching phenotypes (Stirnberg et al., 2002; Turnbull et al., 2002; Sorefan et al., 2003; Booker et al., 2004; Beveridge, 2000; Beveridge et al., 2003; Napoli, 1996; Snowden and Napoli, 2003). For a subset of the mutants (*max1*, *max3*, and *max4*; *rms1*, *rms2*, and *rms5; dad1*), grafting wild-type root stocks to mutant scions restores a wild-type branching habit, indicating that these genes are required for the production of at least one long-range graft-transmissible signal that inhibits shoot branching (Turnbull et al., 2002; Sorefan et al., 2003; Beveridge et al., 1994; Foo et al., 2001; Morris et al., 2001; Napoli, 1996). The reciprocal grafts, with wild-type scions and mutant roots, also have wild-type shoot branching, indicating that the signal(s) can be synthesized in both the root and shoot. The *MAX4* and *MAX3* genes encode divergent members of the carotenoid cleavage dioxygenase family (Sorefan et al., 2003; Booker et al., 2004). MAX3 has been shown to have carotenoid cleaving activity in *E. coli* and MAX4 is able to cleave one of the MAX3 cleavage products (Booker et al., 2004; Schwartz et al., 2004). This is consistent with their proposed role in the synthesis of one or more long-range signals. A close homolog of *MAX4* was isolated from pea and shown to be *RMS1* (Sorefan et al., 2003).

Because of the predicted biochemical activity of MAX3 and MAX4/RMS1, and the measured levels of known branch-regulating hormones in *rms* and *max* mutants, the MAX/RMS-dependent hormone(s) is not a classical plant hormone (Beveridge et al., 1994, 1997, 2000; Morris et al., 2001; Booker et al., 2004). In order to understand better this hormone(s), we have investigated the relationship between the different *MAX* loci. We propose a model in which *MAX1* acts downstream of *MAX3* and *MAX4* in the synthesis of the hormone, which requires MAX2 for its perception. Molecular analysis of the *MAX1* locus is consistent with this model.

*Correspondence: hmol1@york.ac.uk

[4]These authors contributed equally to this work.

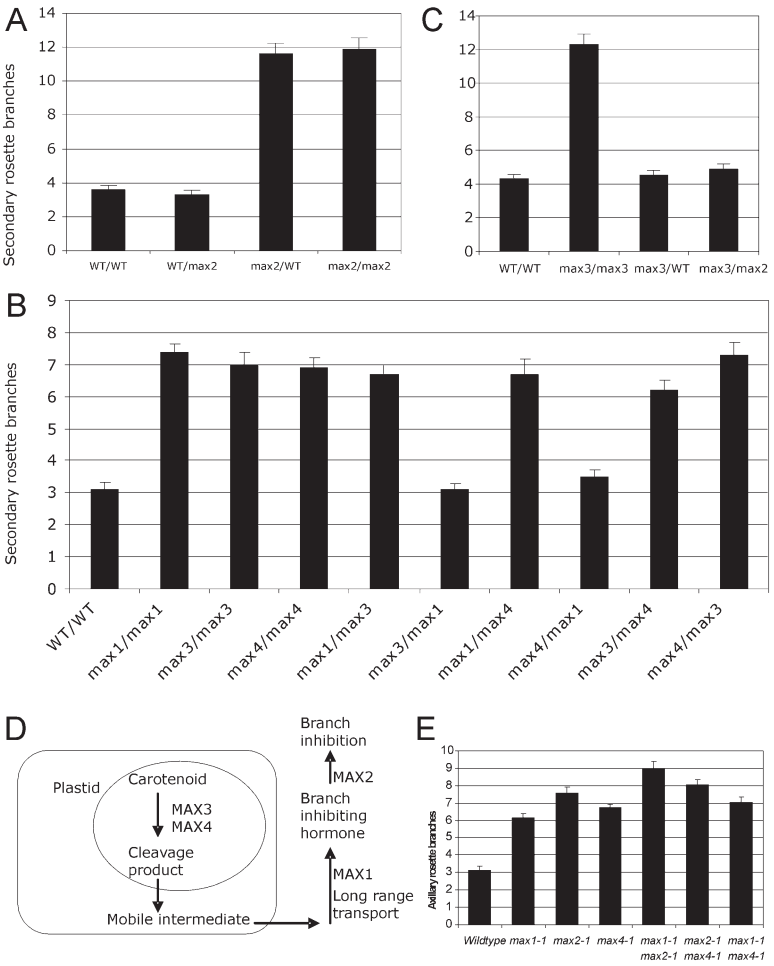

Figure 1. Characterization of the MAX Pathway

Mean number of second-order rosette branches of reciprocal grafts between (A) *max2* and WT, (B) *max3* and *max4*, *max1* and *max3*, and *max1* and *max4*, and (C) *max2* and *max3*. Graft combinations are annotated scion/rootstock. Plants were analyzed 37–40 days after grafting. Error bars represent the standard errors of the means, n = 8–17. (D) Model to show proposed action of *MAX1–MAX4* in a single pathway. (E) Mean number of second-order rosette branches of single- and double-mutant combinations of *max1*, *max2*, and *max4* plants. Branching was scored when the primary apex had ceased activity. Error bars represent the standard errors of the means, n = 12–18.

Results and Discussion

We have previously described the phenotypes conferred by mutation at four *Arabidopsis* loci, *MAX1–MAX4* (Stirnberg et al., 2002; Turnbull et al., 2002; Sorefan et al., 2003; Booker et al., 2004). The phenotypes are very similar and include increased shoot branching and reduced stature, petiole, and leaf blade length. Furthermore, the *MAX1*, *MAX3*, and *MAX4* loci are all required for the production of an upwardly mobile, branch-inhibiting signal or signals. We therefore tested the attractive hypothesis that the *MAX* genes act in a single pathway.

Grafting Experiments Identify Three Types of *max* Mutant

We have already shown that grafting wild-type rootstocks to *max1*, *max3*, or *max4* mutant scions restores a wild-type shoot branching pattern to the mutant shoots (Turnbull et al., 2002; Sorefan et al., 2003; Booker et al., 2004). To determine whether the same is true for *max2*, reciprocal grafts were constructed between *max2* and wild-type, and grown under long-day conditions before scoring the number of axillary branches from the rosette. The *max2*/*max2* controls developed numerous axillary branches from the rosette compared to WT/WT controls (Figure 1A). In both *max2*/WT and WT/*max2* grafts the branching phenotype was the same as the scion genotype (Figure 1A). This suggests that the *MAX2* gene acts in the shoot to inhibit branching.

To determine the relationship between the different *max* mutants, we performed reciprocal grafting experiments between the *max* mutants. Grafting *max3* scions to *max4* rootstocks and vice versa resulted in plants with high levels of branching, similar to the respective *max*/*max* controls (Figure 1B). This suggests that these mutants lack the same signal. Rootstocks from *max3* or *max4* plants were also unable to restore wild-type branching when grafted to *max1* shoots, suggesting that the signal deficient in *max3* and *max4* is the same one that is missing in *max1* mutants. Interestingly, *max1* rootstocks were able to restore wild-type shoot branching patterns when grafted to *max3* and *max4* scions (Figure 1B), which suggests that *max1* rootstocks can produce the signal, or a mobile precursor of the signal, that is deficient in *max3* and *max4*.

To determine the relationship between *MAX2* and the *MAX1/3/4*-dependent signal, reciprocal grafts were carried out between *max2* and *max3*. Rootstocks from *max3* plants had no effect on *max2* scions, while *max2* roots were able to restore wild-type branching to *max3* mutants (Figure 1C). These results indicate that the

MAX2 gene is not required for the synthesis of the *MAX1/3/4*-dependent signal.

Thus, based on the grafting behavior of the mutants, there are three classes of *MAX* gene: *MAX2* acts locally in the shoot and is not required to make the *MAX1/3/4*-dependent signal. *MAX1/3/4* act non–cell autonomously and are required for the synthesis of the same graft-transmissible signal, but *MAX1* and *MAX3/4* differ in that *max1* mutant roots can restore wild-type branching to *max3* and *max4* mutant shoots, though *max3* and *max4* roots cannot restore wild-type branching to *max1* shoots. One explanation for these results is that MAX1, MAX3, and MAX4 act together in the same biosynthetic pathway to produce a branch-inhibiting long-range signal, and MAX2 acts in the perception of this signal in the shoot. The different grafting behavior of *max3*/*max4* and *max1* mutant roots can be explained if MAX3 and MAX4 act earlier in the pathway on nonmobile substrates, while MAX1 acts later on a mobile substrate. Thus, *max3* and *max4* mutant roots do not produce any mobile compound in the biosynthetic pathway, whereas the action of MAX3 and MAX4 in *max1* mutant roots produces a mobile intermediate that can move up the plant and can be converted by MAX1 in the shoot to the active branch-inhibiting signal (Figure 1D). This model is consistent with the molecular identities of MAX3 and MAX4. Both are predicted to be plastidic, a location that has been demonstrated for MAX3 (Booker et al., 2004). Furthermore, in *E. coli*, MAX3 has been shown to cleave a variety of carotenoid substrates, and MAX4 can cleave at least one MAX3 cleavage product (Booker et al., 2004; Schwartz et al., 2004). Thus, they could act on a nonmobile plastidic carotenoid substrate in the synthesis of the branch-inhibiting signal. Similarly, the fact that MAX2 encodes an F-box protein is consistent with its proposed role in signal perception in the shoot (Stirnberg et al., 2002). F-box proteins are a common feature of hormone signal transduction pathways, where they function to target specific regulatory proteins for degradation (Vierstra, 2003).

Elements of this model are similar to that proposed for the RMS loci of pea (Beveridge, 2000; Beveridge et al., 2003). The *rms3* and *rms4* mutants have similar grafting behavior to *max2* and are proposed to act locally in the shoot. The *rms1* and *rms5* mutants have similar grafting behavior to *max3* and *max4*, and are similarly proposed to be required for the production of an upwardly mobile branch-inhibiting signal, and indeed *RMS1* has been shown to be orthologous to *MAX4* (Sorefan et al., 2003). However, in the pea model, *rms2* mutants have similar grafting behavior to *max1*, but the *RMS2* gene is proposed to be required for a second, different, long-distance signal that promotes the synthesis of the RMS1/RMS5-dependent signal in a regulatory loop. *RMS2* is placed in this linked, but distinct pathway because the *rms2* mutants have phenotypes such as wiltiness, not shared by *rms1* and *rms5* mutants, and double mutants between *rms1* and *rms2* have strongly additive branching phenotypes, suggesting that they act at least partly independently (Beveridge et al., 1997). In contrast, the phenotype of *max1* mutants is not readily distinguishable from that of *max3* and *max4* mutants, making a single-pathway model more likely.

Genetic Interactions between the Mutants

To test the single-pathway hypothesis further, we compared the phenotypes of single and double *max1*, *max4*, and *max2* mutants. The number of secondary rosette branches for each genotype was determined after the primary apex had ceased activity. All the single mutants showed similar branching habits, with *max2* being consistently more branched than *max1* or *max4* (Figure 1E). All the double mutant combinations were no more branched than the most branched parent (Figure 1E), with the exception of the *max1-1*/*max2-1* double mutant where a slight increase in branching over the *max2-1* mutant was observed. This increase is not reproducible (Stirnberg et al., 2002). These data lend further support to the single-pathway model (Figure 1D).

Molecular Analysis of the *MAX1* Locus

As described above, the molecular identity of the *MAX3*, *MAX4*, and *MAX2* genes is consistent with their predicted function in the emerging single-pathway model. To determine whether this is also true for *MAX1*, we isolated the gene using a map-based approach. The *max1-1* allele is in the Enkheim-2 ecotype, but for the purposes of phenotypic comparison it was introgressed into the Col background by seven rounds of backcrossing (Stirnberg et al., 2002). Initial mapping revealed close linkage of *MAX1* to the *ERECTA* (*ER*) locus on chromosome 2 (Stirnberg et al., 2002). To determine the position of *max1* with respect to ER, two flanking SSLP markers, F13B15 and F12K2, were used, both about 2.5 cM from ER (Bell and Ecker, 1994). Recombinants were then genotyped for new CAPS markers lying in the interval between F13B15 and F12K2. This located *MAX1* to a 48 kb region between two markers situated on overlapping BAC clones T19L18 and T1D16 (Figure 2A).

From the annotated genes present in this region, At2g26170, a member of the cytochrome P450 family, appeared the strongest contender to act in the biosynthesis of a hormone, and therefore to represent *MAX1*. Transformation of *max1-1* plants with a 4.7 kb genomic fragment containing the At2g26170 open reading frames (ORFs) plus 1,850 bp upstream and 470 bp downstream sequences resulted in full restoration of the wild-type phenotype, suggesting that At2g26170 is *MAX1*. This hypothesis was further supported by the observation that At2g26170 from *max1-1* plants was found to contain a single point mutation (C to T) predicted to convert amino acid 117 from proline to leucine (Figure 2B). In addition, we identified a T-DNA insertional mutant in At2g26170 in the Versailles collection (Figure 2B). Plants homozygous for this insertion were found to have the characteristic *max1* phenotype. Taken together, these data demonstrate that *MAX1* is At2g26170, and its predicted enzymatic function is consistent with a role in the synthesis of the branch-inhibiting signal.

According to *Arabidopsis* cytochrome P450 nomenclature, MAX1 is classified as CYP711A1 and constitutes a single-member family (http://arabidopsis-P450.biotec.uiuc.edu). Analysis of the rice genome revealed the presence of five CYP711 family members (Nelson et al., 2004). Moreover, a close CYP711 homolog was

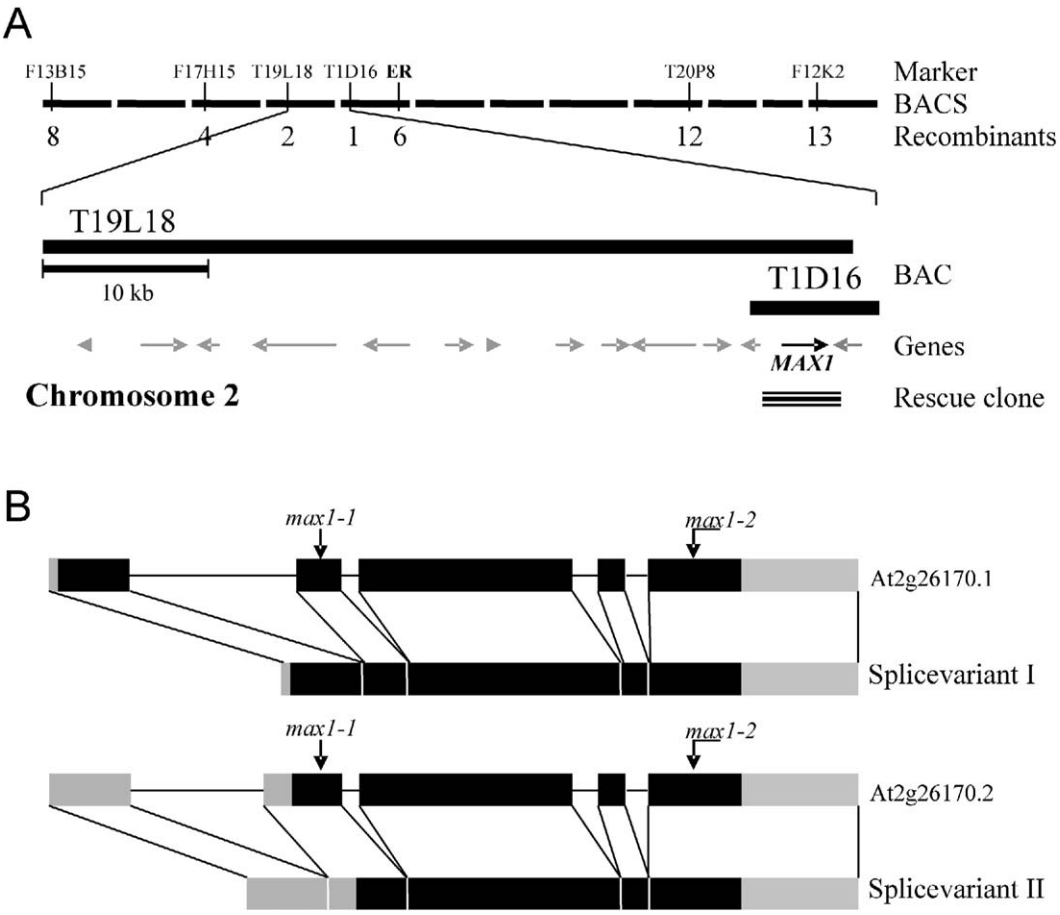

Figure 2. Map-Based Cloning and Database Annotations of the *MAX1* Gene

(A) The *MAX1*-flanking markers that were used to screen for recombinants and the corresponding BAC clones, spanning the genomic region, are indicated at the top. The number of recombinants, identified in a mapping population of 709 individuals, is indicated below each marker. The region between the closest flanking markers (T19L18, T1D16) is enlarged to show the predicted gene structure (arrows) and the localization of the PCR-amplified genomic fragment, which rescued the *max1-1* mutant in the complementation analysis (combined bar). (B) Database annotations of *MAX1* based on cDNA and EST analysis and location of *max1* mutations. The diagram shows the two transcript variants of At2g26170 originating from different 3′ splice site usage in the first intron. Splice variant I (At2g26170.1) contains an ORF of 1,669 bp. Splice variant II (At2g26170.2) results in a shortened ORF of 1,320 bp. Exons are indicated as solid bars, UTRs are in gray, and those segments presumed to be translated are black. Locations of the two *max1* mutations are indicated with an arrow: base substitution in exon 2 in *max1-1* and T-DNA insertion after amino acid 468 (according to At2g26170.1) in *max1-2*.

found in the unicellular green algae *Chlamydomonas reinhardtii*, suggesting wide conservation in the plant kingdom (Nelson et al., 2004).

Members of the plant CYP711 clade, including *MAX1*, show high sequence similarity to Thromboxane A_2 synthase (TXAS), a mammalian cytochrome P450 involved in prostaglandin metabolism (Figure 3). Remarkably, 7 out of 8 amino acid residues known to be involved in heme or substrate binding, and thus essential for TXAS activity (Wang and Kulmacz, 2002), are conserved in the predicted MAX1 amino acid sequence, which has an overall sequence identity of 24% (Figure 3). TXAS catalyzes two distinct reactions with similar rates, namely isomerization and fragmentation of Prostaglandin H_2. TXAS is a class III P450, which does not require molecular oxygen or an electron donor for catalysis. The strong conservation to TXAS suggests that MAX1 is also a class III P450. Plant class III P450s include the plant-specific CYP74 clade (Mansuy, 1998; Werck-Reichhart and Feyereisen, 2000). CYP74s convert fatty acid hydroperoxides into allene epoxides (CYP74A) or cleave them to volatile aldehydes and traumatin (CYP74B). The first reaction is the initial step of the octadecanoid pathway leading to the synthesis of the plant hormone jasmonate, whereas the products of the latter are also involved in wound and defense responses (Feussner and Wasternack, 2002). Interestingly, all the class III P450s described above act on substrates directly generated by dioxygenases. Prostaglandin H_2 is produced by the heme-containing dioxygenase prostaglandin-synthase, while the CYP74 substrates originate from the activity of nonheme iron–containing fatty acid dioxygenases (Werck-Reichhart and Feyereisen, 2000). Thus, the proposed action of MAX1 downstream of the MAX3 and MAX4 dioxygenases is an established pattern for class III P450s.

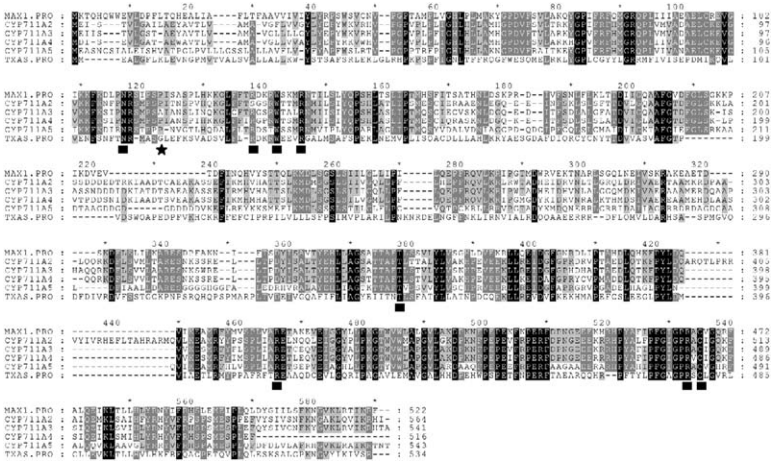

Figure 3. Alignment of Deduced Amino Acid Sequences of MAX1, Four Rice Orthologs, and Human TXAS

MAX1 sequence (GenBank accession number NP_565617); the full-length sequences of four out of five currently known rice orthologs (cultivar japonica) are available from the public database http://drnelson.utmem.edu/rice.html. CYP711A2 (NP_917096), CYP711A3 (NP_917099), CYP711A4 (NP_917100), and CYP711A5 (BAD17629); human TXAS sequence (AAH41157). Black box with white letter, 100% identity; dark gray box with white letter, 80% identity; gray box with black letter, 60% identity. Dashes indicate gaps in the alignment. Amino acid residues shown to be essential for TXAS function and conserved in the MAX1 sequence are underlined with a black bar. The proline residue in MAX1 converted to leucine in *max1-1* is marked with an asterisk (below the alignment).

Expression of the *MAX1* Gene

The public *Arabidopsis* genome databases present two different annotations for MAX1 based on the isolation of two types of cDNAs and ESTs which apparently originate from alternative 3′ splice site usage within the first intron. At2g26170.1 encodes a predicted protein of 522 amino acids, whereas At2g26170.2 results in an N-terminally truncated ORF of 439 amino acids (Figure 2B). We isolated *MAX1* cDNAs from different mRNA sources which all coded for the long version of the protein. Thus, we could not confirm the existence of splice variant At2g26170.2. Our inability to detect this transcript probably resulted from its low expression level or stability or an expression pattern restricted to specific tissues. Our data suggest that the At2g26170.1 splice variant represents the predominant transcript of the MAX1 gene.

To assess the tissue distribution of MAX1 transcription, we screened Affimetrix microarray datasets publicly available from the NSF 2010 homepage (http://arabidopsis-p450.biotec.uiuc.edu/microarray.shtml). This data mining revealed ubiquitous *MAX1* expression. To characterize the sites of *MAX1* transcription in more detail, we generated a promoter-GUS reporter construct (*MAX1::GUS*), using 2 kb of genomic upstream sequence of the MAX1 translational start site (according to At2g26170.1).

The major site for *MAX1::GUS* expression is in vascular-associated tissue throughout the plant (Figure 4). In roots, *MAX1::GUS* activity is restricted to the vascular cylinder (including the pericycle) and starts above the differentiation zone of the meristem (Figures 4A, 4B, and 4G). In inflorescence stems the reporter is detected in the cambial region of vascular bundles, showing some variation in extending either to the xylem or phloem part of the organ (Figure 4F). In axillary regions of leaves and flowers (Figures 4D and 4E) *MAX1::GUS* activity appears to be pronounced, probably due to the presence of vascular junctions. Interestingly, this pattern does not overlap with the known sites for *MAX4* expression, in the root tip and, more weakly and variably, in cortical tissues of the root, shoot, and hypocotyls (Sorefan et al., 2003). These nonoverlapping patterns are consistent with the action of MAX1 downstream of MAX3 and MAX4, on a mobile substrate. Because the mobile signal apparently only moves up the plant (Turnbull et al., 2002), it is tempting to speculate that it travels in the xylem. The expression of *MAX1* in tissue sur-

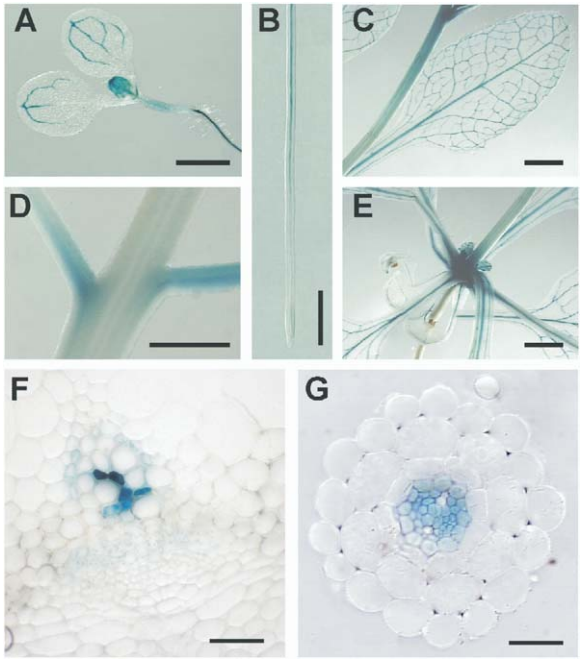

Figure 4. Localization of *MAX1::GUS* Expression

MAX1::GUS activity in the shoot (A) and root (B) of 7-day-old seedlings. Leaf (C), flower-inflorescence stem junctions (D), and rosette region (E) of 25-day-old plants. (F) Cross-section of a vascular bundle from a GUS-stained inflorescence stem (30-day-old plants). *MAX1::GUS* activity is found in the cambial region and xylem-associated parenchyma. (G) Cross-section of a primary root (10 mm distal from the root tip) derived from a 7-day-old seedling. Scale bars represent (A) 2 mm, (B) 0.5 mm, (C–E) 5 mm, and (F and G) 50 μm.

rounding the xylem would allow it to act on a mobile substrate being loaded into or unloaded from the xylem.

We have provided evidence that the *MAX* genes *MAX1–MAX4* act in a single pathway to regulate the synthesis of and response to a nonclassical branch-inhibiting hormone. The predicted function and expression pattern of the *MAX1* gene are consistent with its proposed position in this pathway, downstream of *MAX3* and *MAX4* in the synthesis of the hormone. Our priority now is to identify the hormone to allow more direct tests for this model.

Experimental Procedures

Plant Growth Conditions

Plants were grown in 4 cm square compartments (P40; Cookson Plantpak, Maldon, UK) on F2 compost treated with Intercept (both Levington Horticulture, Ipswich, UK). Plants were grown under long-day conditions (16 hr light at 20°C and 8 hr darkness at 15°C). When the primary apex had ceased growing, shoot branching was scored according to Talbert et al. (1995).

Grafting

Grafting was carried out as described in Turnbull et al. (2002).

Construction of Double Mutants

max1-1/max2-1 was constructed as described in Stirnberg et al. (2002). The *max2-1/max4-1* double mutant was constructed by screening the F2 progeny for individuals showing the elongated hypocotyl, characteristic of *max2-1* homozygotes. *max4-1* homozygotes were identified among the *max2-1* homozygotes by screening their progeny for 100% BASTA-resistant individuals, a characteristic of the transposon-induced *max4-1* allele. *max1-1/max4-1* double mutants were isolated by identifying plants with the *max* phenotype in the F2, but whose progeny segregated for BASTA resistance. These plants were therefore *max1-1* homozygous and *max4-1* heterozygous. F4 seed from these F2 plants were screened to identify *max4-1* homozygotes.

Map-Based Cloning of *MAX1*

F2 mutant progeny from a cross between *max1-1* (BC7 Col) and wild-type Columbia ecotype were used as a mapping population. Plants, recombinant between *MAX1* and the two flanking SSLP markers F13B15 or F12K2, were genotyped for new CAPS markers (http://www.arabidopsis.org) located in this genomic interval. CHAPS markers which delimited MAX1 to a 48 kb chromosomal region were ww443676 (BAC clone T19L18) and ww443819 (BAC clone T1D16). Marker T19L18 ww443676 is a HaeIII polymorphism in a 385 bp PCR product amplified with oligonucleotides 5′-TGA GACGGCACCTTTTTACA-3′ and 5′-TCCGTCCAAAGAACAACACC-3′. T1D16 ww443819 is an AluI polymorphism in a 752 bp PCR product amplified with oligonucleotides 5′-GTTCGAACGCTTGT GACTGA-3′ and 5′-GCAGCTGGAGAGAGAAGCAT-3′.

The 4.7 kb genomic fragment used for complementation analysis was amplified by PCR using oligonucleotides 5′-ACTACTCTC TTCTCCACTCTTGCAG-3′ and 5′-CATGGGAGACTAGACTTATGGA CAC-3′ and cloned into the plant transformation vector pCambia-2300 (GenBank accession number AF234315) using the endogenous restriction sites XbaI (5′) and SacI (3′). The resulting construct was brought into *max1-1* mutant plants by Agrobacterium-mediated transformation (Clough and Bent, 1998).

Promoter-Reporter Fusion Constructs and Reporter Detection

For construction of *MAX1::GUS*, we amplified 2,012 bp of MAX1 promoter sequence from Col genomic DNA using oligonucleotides 5′-GCAAGCTTGTCTAAGGCA-TAGACTTGTC-3′ and 5′-GCGGATC CCTCTAACCTCTAAAGTTCTC-3′. The resulting fragment was cloned into pCR2.1 (Invitrogen, Paisley, UK). This promoter fragment was excised from pCR2.1 using HindIII and BamHI and ligated into HindIII/BamHI-cut pBI 101.3 (Clontech, Palo Alto, CA) in front of the promoter-less GUS ORF, to give pMAX1::GUS.

Col plants were transformed with pMAX1::GUS. T2 progeny of several independent transformants were tested for GUS staining and a representative line containing a single transgene was brought to homozygosity and subsequently used for detailed analysis. GUS staining was performed as described (Jefferson et al., 1987). For cross-sections, GUS-stained material was subjected to 20%, 35%, and 50% ethanol, fixed with FAA (50% ethanol, 10% glacial acetic acid, 5% formaldehyde), and further dehydrated in an ethanol series (70%, 80%, 90%, 95%, 100%), each step for a period of 30 min. Samples were embedded in Technovit 7100 (Heraeus, Wehrheim, Germany) and 12 μm sections were examined with a Nikon OPTIPHOT microscope (Nikon, Kawasaki, Japan).

Acknowledgments

We thank Stephen Day for critical reading of the manuscript and the horticultural technicians at York for plant care. This work was supported by Yara International, Norway; the Biotechnology and Biological Sciences Research Council of the UK; and Schroedinger Fellowship J2346-B12 (T.S.) from the FWF, Austria.

Received: November 18, 2004
Revised: December 23, 2004
Accepted: January 4, 2005
Published: February 28, 2005

References

Bangerth, F. (1994). Response of cytokinin in the xylem exudates of bean (Phaseolus vulgaris L.) plants to decapitation and auxin treatment, and relationship to apical dominance. Planta *194*, 439–442.

Bell, C.J., and Ecker, J.R. (1994). Assignment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics *19*, 137–144.

Beveridge, C.A. (2000). Long-distance signaling and a mutational analysis of branching in pea. Plant Growth Regul. *32*, 193–203.

Beveridge, C.A., Ross, J.J., and Murfet, I.C. (1994). Branching mutant *rms-2* in *Pisum sativum*, branching studies and endogenous indole-3-acetic acid levels. Plant Physiol. *104*, 953–959.

Beveridge, C.A., Symons, G.M., Murfet, I.C., Ross, J.J., and Rameau, C. (1997). The *rms1* mutant of pea has elevated indole-3-acetic acid levels and reduced root-sap zeatin riboside content but increased branching controlled by graft-transmissible signals. Plant Physiol. *115*, 1251–1258.

Beveridge, C.A., Symons, G.M., and Turnbull, C.G.N. (2000). Auxin inhibition of decapitation-induced branching is dependent on graft-transmissible signals regulated by genes *Rms1* and *Rms2*. Plant Physiol. *123*, 689–697.

Beveridge, C.A., Weller, J.L., Singer, S.R., and Hofer, J.M.I. (2003). Axillary meristem development: budding relationships between networks controlling flowering, branching and photoperiod responsiveness. Plant Physiol. *131*, 927–934.

Booker, J.P., Chatfield, S.P., and Leyser, O. (2003). Auxin acts in xylem-associated or medullary cells to mediate apical dominance. Plant Cell *15*, 495–507.

Booker, J., Auldridge, M., Wills, S., McCarty, D., Klee, H., and Leyser, O. (2004). MAX3/CCD7 is a carotenoid cleavage dioxygenase required for the synthesis of a novel plant signaling molecule. Curr. Biol. *14*, 1232–1238.

Chatfield, S.P., Stirnberg, P., Forde, B.G., and Leyser, O. (2000). The hormonal regulation of axillary bud growth in *Arabidopsis*. Plant J. *24*, 159–169.

Cline, M.G. (1991). Apical dominance. Bot. Rev. *57*, 318–358.

Clough, S.J., and Bent, A.F. (1998). Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J. *16*, 735–743.

Feussner, I., and Wasternack, C. (2002). The lipoxygenase pathway. Annu. Rev. Plant Biol. *53*, 275–297.

Foo, E., Turnbull, C., and Beveridge, C.A. (2001). Long distance signaling and the control of branching in the *rms1* mutant of pea. Plant Physiol. *126*, 203–209.

Jefferson, R.A., Kavanagh, T.A., and Bevan, M.W. (1987). GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J. *6*, 3901–3907.

Leyser, O. (2003). Regulation of shoot branching by auxin. Trends Plant Sci. *11*, 541–545.

Mansuy, D. (1998). The great diversity of reactions catalyzed by cytochromes P450. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. *121*, 5–14.

Morris, D.A. (1977). Transport of exogenous auxin in two-branched dwarf pea seedlings (*Pisum sativum* L.). Planta *136*, 91–96.

Morris, S.E., Turnbull, C.G.N., Murfet, I.C., and Beveridge, C.A. (2001). Mutational analysis of branching in pea. Evidence that *Rms1* and *Rms5* regulate the same novel signal. Plant Physiol. *126*, 1205–1213.

Napoli, C.A. (1996). Highly branched phenotype of the petunia *dad1-1* mutant is reversed by grafting. Plant Physiol. *111*, 27–37.

Nelson, D.R., Schuler, M.A., Paquette, S.M., Werck-Reichhart, D., and Bak, S. (2004). Comparative genomics of rice and *Arabidopsis*. Analysis of 727 cytochrome P450 genes and pseudogenes from a monocot and a dicot. Plant Physiol. *135*, 756–772.

Nordstrom, A., Tarkowski, P., Tarkowska, D., Norbaek, R., Astot, C., Dolezal, K., and Sandberg, G. (2004). Auxin regulation of cytokinin biosynthesis in *Arabidopsis thaliana*: a factor of potential importance for auxin-cytokinin-regulated development. Proc. Natl. Acad. Sci. USA *101*, 8039–8044.

Schwartz, S.H., Qin, X., and Loewen, M.C. (2004). The biochemical characterization of two carotenoid cleavage enzymes from *Arabidopsis* indicates that a carotenoid-derived compound inhibits lateral branching. J. Biol. Chem. *279*, 46940–46945.

Snowden, K.C., and Napoli, C. (2003). A quantitative study and lateral branching in petunia. Funct. Plant Biol. *30*, 987–994.

Sorefan, K., Booker, J., Haurogne, K., Goussot, M., Bainbridge, K., Foo, E., Chatfield, S., Ward, S., Beveridge, C., Rameau, C., and Leyser, O. (2003). MAX4 and RMS1 are orthologous dioxygenase-like genes that regulate shoot branching in *Arabidopsis* and pea. Genes Dev. *17*, 1469–1474.

Stirnberg, P., van De Sande, K., and Leyser, H.M.O. (2002). *MAX1* and *MAX2* control shoot lateral branching in *Arabidopsis*. Development *129*, 1131–1141.

Talbert, P.B., Adler, H.T., Parks, D.W., and Comai, L. (1995). The *REVOLUTA* gene is necessary for apical meristem development and for limiting cell division in the leaves and stem of *Arabidopsis thaliana*. Development *121*, 2723–2735.

Turnbull, C.G.N., Booker, J.P., and Leyser, H.M.O. (2002). Micrografting techniques for testing long-distance signalling in *Arabidopsis*. Plant J. *32*, 255–262.

Vierstra, R.D. (2003). The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. Trends Plant Sci. *8*, 135–142.

Wang, L.H., and Kulmacz, R.J. (2002). Thromboxane synthase: structure and function of protein and gene. Prostaglandins Other Lipid Mediat. *68–69*, 409–422.

Werck-Reichhart, D., and Feyereisen, R. (2000). Cytochromes P450: a success story. Genome Biol *1*, reviews 3003.1–3003.9.