MAX1, a regulator of the flavonoid pathway, controls vegetative axillary bud outgrowth in *Arabidopsis*

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We show that *MAX1*, a specific repressor of vegetative axillary bud outgrowth in *Arabidopsis*, acts a positive regulator of the flavonoid pathway, including 11 structural genes and the transcription factor *An2*. Repression of bud outgrowth requires *MAX1*-dependent flavonoid gene expression. As the flavonoidless state leads to lateral outgrowth in *Arabidopsis*, our data suggest that a flavonoid-based mechanism regulates axillary bud outgrowth and that this mechanism is under the control of *MAX1*. Flavonoid gene expression results in the diminished expression of auxin transporters in the bud and stem, and this, in turn, decreases the rate of polar auxin transport. We speculate that *MAX1* could repress axillary bud outgrowth via regulating flavonoid-dependent auxin retention in the bud and underlying stem. Because *MAX1* is implicated in synthesis of the carotenoid-derived branch regulator(s) from the root, it likely links long-distance signaling with local control of bud outgrowth.

branching | apical dominance

Regulation of axillary bud outgrowth has been of major interest for more than 70 years. Initial focus on the hormonal regulation of the process revealed that auxin from the apical meristem is central to bud repression, laying the foundation for one of the oldest paradigms of plant biology, namely, apical dominance (1). A subsequent unexpected finding that auxin from the apex does not enter the bud (2) suggested that another long-range signal in the control of lateral branching was involved and led to the discovery of cytokinine transported from the root (3). Recent studies with the *ramosus* series of shoot branching mutants (*rms1–rms5*) in pea revealed that there is still another long-distance signal originating in the root that negatively affects branching (4, 5). Graft rescue of *max1* (6), *max3* (7), and *max4* (8) mutants from *Arabidopsis* further verified the existence of a graft transmissible branch-inhibiting signal from the root and demonstrated that this element of the basic control mechanisms also is conserved. *MAX4* has homology to carotenoid cleavage dioxygenases, but its biochemical activity has not been established (8). *MAX3* encodes CCD7, a carotenoid cleavage dioxygenase, and is required for synthesis of a carotenoid-derived long-range signal in the control of shoot branching (7). *MAX2* is an F-box leucine-rich repeat-containing member of the SCF family of ubiquitin ligases (9) proposed to play a role in the perception of the *MAX1*-, *MAX3*-, and *MAX4*-dependent signal in the stem (10). *MAX1* acts downstream of *MAX3* and *MAX4* in the same pathway and encodes a member of the CYP450 family, CYP711A1, with high similarity to the mammalian thromboxane synthase (11), but direct evidence for its enzymatic function is still missing.

In *Arabidopsis*, there is strong evidence that flavonoids control polar auxin transport in the stem (12), regulate expression of auxin efflux genes at the steady-state mRNA level, and effect subcellular localization of efflux proteins in the root (13). Studies of flavonoid pathway mutants in *Arabidopsis* indicate that a change in flavonoid profile, level and/or distribution in the tissues can lead to redistribution of auxin efflux carriers, which in turn change local auxin levels and cell fate (13).

Here we provide evidence that *MAX1* is a specific repressor of vegetative bud outgrowth and that it positively regulates the flavonoid pathway in the axillary bud and stem. Flavonoid gene expression inversely correlates with polar auxin transport and with the expression of a set of auxin influx and efflux carriers in the stem and bud. We propose a model in which flavonoid-dependent auxin retention in the stem and bud under the control of *MAX1* is an essential element of the regulatory mechanism for bud repression.

Materials and Methods

For a detailed version of this section, see *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

DNA Microarray Analysis. Total RNA was isolated from the "trunk" (defined here as the rosette stem together with ≈0.5 mm of the petioles and axillary buds) of late vegetative *Arabidopsis* plants grown at 23°C with a 13-h photoperiod and at 60% humidity. Total RNA was pooled from ≈80 plants. Affymetrix *Arabidopsis* GeneChip processing was done in the core facility of the Brigham and Woman Hospital, Boston. Gene expression analysis was performed from a single set of hybridization by using the RESOLVER 4.0 software package (Rosetta Biosoftware, Seattle, WA), with the cutoff set at 2-fold change and with log10 intensity 0.0 or above at $P = 0.05$. To verify DNA microarray data, RNA blots (from total RNA as above) were hybridized with RT-PCR-generated, gene-specific probes, as specified for the DNA array in the manufacturer's instructions (Affymetrix). This approach proved useful to identify potential downstream targets for *max1* but could not support a statistical analysis.

Flavonoid Staining. Hand-sectioned rosette-region stem was stained for 15–20 min with saturated 0.25% (wt:vol) diphenylboric acid-2-aminoethyl ester with 0.02% (vol:vol) Triton X-100 and viewed with an epifluorescent microscope with a FITC filter (excitation, 450–490 nm; suppression, long pass/515 nm) according to Murphy *et al.* (14).

Chemical Complementation of *max1-3*. For each treatment, 25 plants were grown until the late vegetative stage at 23°C and with 60% relative humidity, a 16-h photoperiod, and at ≈60 μE $cm^{-2}{\cdot}s^{-1}$ (μE = microeinstein) light intensity. Starting 7 days before bolting, the rosette leaf axils, together with the rosette stem, were treated daily with 50 μl of 10 mM naringenin, kempferol, or quercetin in H_2O at pH 9.3 containing 0.02% Silwet L-77 (Lehle seeds) as a wetting agent. At day 7, the dose was increased to 100 μl per plant daily for another 7 days. Lateral outgrowth was scored 1 week after the last treatment.

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Abbreviations: IAA, indole-3-acetic acid; NPA, naphthylthalamic acid; wt, wild type.

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Control plants were treated with H_2O at pH 9.3 containing 0.02% Silwet L-77.

Measurements of Polar Auxin Transport. Polar auxin transport was measured in the 30-mm-long rosette proximal stem segment that included the trunk by using a modification of the procedure described by Brown *et al.* (12). Briefly, single stem segments were placed in a 500-μl tube with the apical end submerged in 20 μl of buffer [5 mM MES/1% (wt:vol) sucrose/500 pM cold indole-3-acetic acid (IAA)/0.3 μl of 37 MBq/ml 3-[5(n)-^{3}H]IAA, pH 5.5 (Amersham Biosciences, which is now GE Healthcare)], in the presence or absence of 10 μM NPA. Segments were incubated for ≈3 h at room temperature in the dark and dissected into 5-mm-long sections, and the amounts of radioactivity in the sections were measured in a liquid scintillation counter after 3 days of incubation at ambient temperature. The data reported are the averages ± SEM of three segments per treatment.

Results

From a screen for branching mutants of T-DNA insertion lines of *Arabidopsis*, we have isolated a knock-out mutant of the recently described *MAX1* gene (see *Supporting Materials and Methods*; see also Fig. 4, which is published as supporting information on the PNAS web site) (11). An F2 segregant from the backcross to the C24 ecotype was designated *max1-3* and was used for further analysis. *max1-3* displayed increased branching, rounder rosette leaves, smaller rosette diameter, and reduced stature, a phenotype similar to those of the other recessive axillary bud outgrowth *max* mutants of *Arabidopsis* (8, 9, 11). Ectopic overexpression of *MAX1* eliminated bud outgrowth in the rosette region stem but had no effect on the higher order branching (see *Supporting Materials and Methods* and Fig. 4). Therefore, *MAX1* is a specific repressor of vegetative axillary buds generated by the axillary meristem. Expression of *MAX1* in vascular traces in the rosette stem and axillary buds throughout plant development (see Fig. 5, which is published as supporting information on the PNAS web site) is consistent with this function.

Flavonoids Are Essential Regulators of Vegetative Bud Outgrowth. To study the genetic network(s) downstream of *MAX1* that control repression of bud outgrowth, the gene expression profile of the late vegetative stage rosette stem was examined together with ≈0.5 mm of the petioles and axillary buds (trunk). We identified 63 down-regulated and 27 up-regulated genes in *max1-3* (data not shown). The DNA microarray data revealed an intriguing coordinated down-regulation of 11 structural genes (*CHS*, *CHI*, *F3H*, *F3′H*, *FLS*, *DFR*, *ANS*, *UFGT*, *RT*, *AAC*, and *GST*) and the transcription activator *An2*, all of which are in the flavonoid pathway. These data were verified by RNA blots (Fig. 1*A*) and RT-PCR (Fig. 1*B*). Only the RT-PCR data for An2, which is not detectable at the resolution of the RNA blot, is shown in Fig. 1*B*.

Loss of apical dominance in the flavonoidless state in the *CHS tt4* mutant (12) and the strong apical dominance in gain-of-function plants overexpressing the An2/PAP1 transcription activator (15) indicated an inverse correlation between flavonoid gene expression and lateral growth. This correlation was confirmed by using light-stress induction of flavonoid genes that resulted in anthocyanin accumulation and strong apical dominance in the wild type (wt); whereas, in *max1-3*, the lack of flavonoid gene induction remained tightly coupled with the lack of repression in axillary bud outgrowth (Fig. 1 *A* and *C*). These data suggest that there could exist a flavonoid-based mechanism in the trunk that plays a role in the repression of axillary bud outgrowth and that this mechanism is under the control of *MAX1*.

***MAX1* Is Essential for Flavonoid Gene Expression in the Axillary Bud and Underlying Stem.** The trunk was dissected, and the expression in the bud, stem, and apex of the "early" genes (*CHS*, *F3H*, *FLS*, and *F3′H*) and the "late" gene, *DFR*, were compared. High steady-state RNA levels in the bud and low levels in the stem for *FLS* and *F3′H* indicate that flavonoid production is predominantly localized to the repressed bud in the wt plant (Fig. 1*D*). In *max1-3*, the overall steady-state mRNA levels of flavonoid genes (with the exception of *CHS*) were reduced ≥5-fold in each organ. It is possible, however, that the lowered expression levels of flavonoid genes in outgrowing buds of the mutant compared with repressed wt buds might be affected by differences in bud outgrowth affecting the composition of the sampled tissues.

Repression of Bud Outgrowth Requires a Switch from *MAX1*-Independent to *MAX1*-Dependent Flavonoid Gene Expression During Bud Formation. The dominant flavonoids in *Arabidopsis* are glycosides and aglycons of kempferol and quercetin (16). However, there has not yet been a comprehensive analysis of the flavonoid profile in the axillary bud and stem. An analysis of the spatial distribution of flavonoids using the flavonoid-specific histochemical stain diphenylboric acid-2-aminoethyl ester (14, 16) in cross-sectioned trunks showed accumulation in the repressed buds of wt (Fig. 1*Ea* and *Eb*). The bright signal in *max1-3* buds during bud formation (Fig. 1*Ec*), at least in part, may derive from yellow staining naringenin produced early in the pathway (Fig. 1*H*), consistent with the expression of *CHS* and decreased *F3H* and *FLS* production (Fig. 1*D*). In contrast, in the outgrowing axillary buds of the mutant, diphenylboric acid-2-aminoethyl ester staining diminished significantly (Fig. 1*Ed*), and there was no visually detectable increase in the stainable flavonoids in the stem. These findings are consistent with the reduced flavonoid gene expression in *max1-3* (Fig. 1*D*).

To determine the timing of *MAX1*-dependent expression of the flavonoid pathway genes, expression profiles were analyzed during early bud formation in the vegetative stage. Before bud initiation, flavonoid gene transcript levels were comparably high in *max1-3* and wt trunk (data not shown), but, during bud formation (≤0.5-mm buds), these transcripts already showed significant differences (Fig. 1*F*). Thus, histological staining and gene expression profiles support a central role of *MAX1* in the regulation of the flavonoid pathway in the axillary bud (Fig. 1*H*).

Exogenous Application of Quercetin Rescues the *max1* Phenotype. To test whether flavonoids can repress bud outgrowth, flavonoids were applied to late vegetative stage buds and stem of *max1-3*. Treatment with 1 mM of exogenous kempferol and quercetin resulted in a subtle reduction in lateral outgrowth that was difficult to assess. Because aglycon flavonoids have poor permeability (17), we increased the flavonoid concentration to 10 mM at pH 9.3. Solution behavior of flavonoids at high concentrations is highly complex and shows reduced stability at this pH (18). However, this treatment (from most to least effective: quercetin > kempferol > naringenin) was sufficient to partially reverse the bud outgrowth in *max1-3* as assessed by rosette leaf formation (Fig. 1*G*). In addition, quercetin and kempferol reduced the number of laterals to 6.7 ± 1.4 and 8.2 ± 2, respectively; whereas naringenin was relatively ineffective, resulting in 11.6 ± 2.9 laterals versus the 13.3 ± 2.5 laterals in the mock-treated control. This result is not due to toxicity of flavonoid oxidative byproducts or to nonspecific functions of the high concentration of the compounds because treatment of the wt axillary buds was not inhibitory to growth and development (data not shown). *max1-3* bud repression can then be explained as complementation of the deficiency by an exogenous flavonoid. This interpretation is also consistent with the gene-expression-based prediction that the endogenous flavonoid level in the mutant is too low to prevent bud outgrowth.

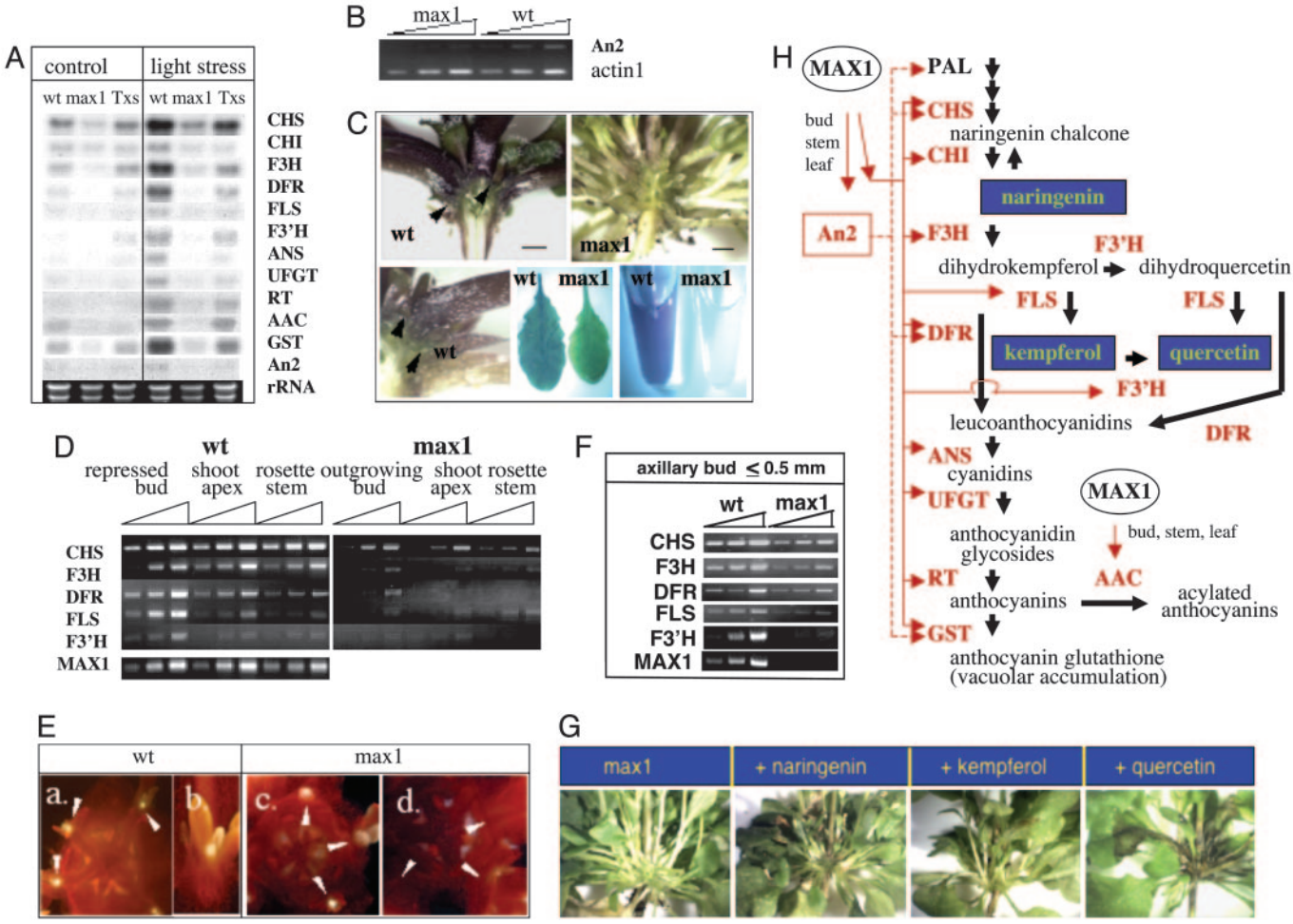

Fig. 1. *MAX1* is a positive regulator of the flavonoid pathway in the late vegetative stage plant. (*A*) RNA blot analysis of flavonoid pathway genes in late vegetative stage trunk. The plant genotypes are wt, loss-of-function mutant max1 (*max1-3*), and gain-of-function transgenic (Txs). Plants were grown under normal conditions (control) or exposed to light stress (≈160 μE cm^{-2}·s^{-1}) for 2 days. Flavonoid pathway genes, with locus tags in parentheses, are as follows: *CHS*, chalcone synthase (At5g13930); *CHI*, chalcone isomerase (At5g05270); *F3H*, flavanone 3-hydroxylase (At3g51240); *DFR*, dihydroflavonol 4-reductase (At5g42800); *FLS*, flavonol synthase (At5g08640); *F3′H*, flavonoid 3′ hydroxylase (At5g07990); *ANS*, anthocyanidin synthase (At4g22870); *UFGT*, *UDP* glucose:flavonoid 3-O-glucosyltransferase (At5g17050); *RT*, *UDP* rhamnose-anthocyanindin-3-glucoside rhamnosyltransferase (At4g27570); *AAC*, anthocyanin 5-aromatic acyltransferase (At1g03495); *GST*, glutatione *S*-transferase (At5g17220); *An2*, anthocyanin 2 (At1g56650). (*B*) A semiquantitative RT-PCR analysis of *An2*/*PAP1* expression in late-vegetative-stage trunk using 0.5, 1.0, and 2.0 μl of the reverse transciption reaction as template; actin-1 is the internal control. (*C*) Light stress response under continuous illumination after 9 days. Longitudinal sections of rosette region stem of wt (*Upper Left* and *Lower Left*) and *max1-3* (*Upper Right*); repressed axillary buds are marked by arrowheads. (*Lower Center*) wt and *max1-3* leaves. (*Lower Right*) Extractable anthocyanins from uppermost rosette leaves (500 mg of fresh weight). (Scale bar, 1 mm.) (*D*) Expression profiles of flavonoid pathway genes in the dissected trunk of late-vegetative-stage plants. Semiquantitative RT-PCR was performed as described for *B*. (*Ea–Ed*) Flavonoid staining. Cross sections of the vegetative rosette stem were stained with diphenylboric acid-2-aminoethyl ester and viewed through a FITC filter. Flavonols (kempferol and quercetin) stain orange, naringenin stains yellow, sinapate esters are green, and chlorophyll autofluorescence is red. White arrows indicate axillary buds before (*a–c*) and during bud outgrowth (*d*). (*F*) Expression of flavonoid pathway genes in axillary buds (<0.5 mm). Semiquantitative RT-PCR was used as described for *D*. (*G*) Chemical complementation of *max1-3* using exogenous application of flavonoid pathway intermediates: naringenin, kempferol, and quercetin. Axillary buds and rosette stem were treated daily for 2 weeks, starting 7 days before bolting. Plants were grown for another week before documentation. (*H*) A model for *MAX1*-regulated flavonoid gene expression in the late-vegetative-stage plant. Genes that require *MAX1* for normal level expression are red. *An2* controls at least four genes of the pathway (15), as shown by dotted lines. Pathway intermediates used in the chemical complementation of *max1-3* are highlighted.

Flavonoid Gene Expression Inversely Correlates with the Expression of Auxin Transporters and with the Rate of Polar Auxin Transport in the Inflorescence Stem. Because quercetin is known to interact with the auxin transport mechanism (19), we hypothesized that change in the flavonoid level might alter auxin movement in *max1-3*. The representative data set shown in Fig. 2*A* describes the movement of [^{3}H]IAA through 5-mm-long consecutive sections of the rosette proximal stem segment during a 190-min transport period. The synthetic auxin transport inhibitor NPA (10 μm) similarly reduced [^{3}H]IAA movement in wt, *max1-3*, and Txs to the first 5-mm submerged end and adjacent section (Fig. 2*A*, lower bar graph), suggesting that most of the auxin movement measured in the assay (Fig. 2*A*, upper bar graph) is due to active transport. However, compared with wt, for which [^{3}H]IAA transport peaked at the fourth stem section, the auxin peak in *max1-3* was in the sixth section and the amount of [^{3}H]IAA in the peak was higher than wt. These results were reproducible in separate experiments but with different absolute values, and they demonstrated that auxin moves with an elevated rate and volume in *max1-3* compared with the wt.

An RNA blot analysis of auxin transporter gene expression in stem segments of seed-setting-stage plants indicated a *MAX1*-dependent expression profile (Fig. 2*B*) that was consistent with the

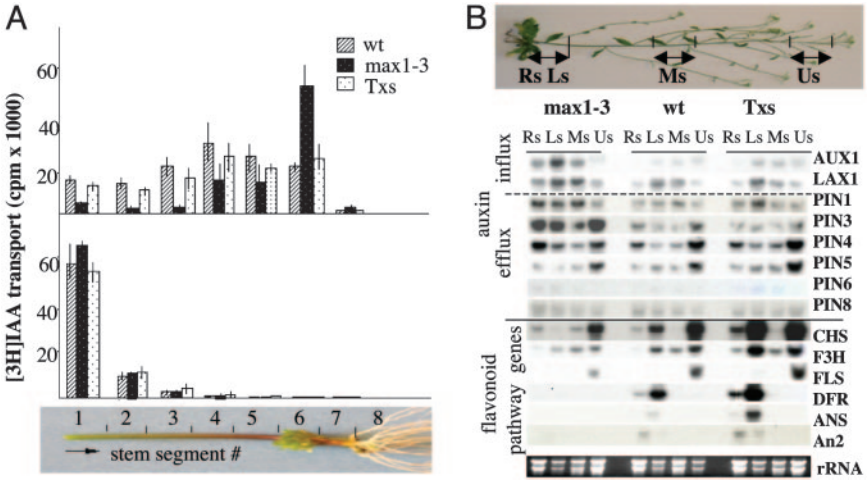

Fig. 2. Polar auxin transport and expression of auxin transporter and flavonoid genes in the inflorescence stem. (*A*) Comparison of [^{3}H]IAA transport in the rosette proximal inflorescence stem segment of wt, loss-of-function (*max1-3*), and gain-of function (Txs) plants without (upper bar graph) and with NPA (lower bar graph). Segment 6 is the trunk. Data are the average ± SE of three segments. (*B*) An RNA blot analysis of spatial expression of auxin transporter and flavonoid genes in seed-setting-stage stem. Ethidium bromide-stained gel is shown below as a loading control. (*Upper*) Stem segments included the trunk, which encompassing the rosette stem with the axillary buds and base of petioles (Rs), the lower stem (Ls), the middle stem (Ms), and the upper stem (Us). The auxin transporter genes, with locus tags in parentheses are *AUX1* (At2g21050), *LAX1* (At 5g01240), *PIN1* (At1g73590), *PIN3* (At1g70940), *PIN4* (At2g01420), *PIN5* (At1g23080), *PIN6* (At1g77110), and *PIN8* (At5g16530).

auxin transport data. The *max1-3* mutation dramatically increased expression of the auxin influx genes *AUX1*-like and *LAX1* and the *PIN1*, *PIN3*, and *PIN4* auxin efflux facilitator genes, primarily in the trunk and in the rosette proximal stem segments (Fig. 2*B*, compare lanes Rs and Ls in *max1-3* with those for wt and Txs). These changes contrasted with the relative stability of the expression profile in the gain-of-function transgenic (Fig. 2*B*, compare wt with Txs), indicating a lack of additive effects and suggesting that the increase in auxin transporter RNA level in *max1-3* could possibly be due to a lack of repression.

Flavonoid mutants of *Arabidopsis* also display altered auxin transport and altered expression of PIN family genes (13). To determine whether the increased auxin transporter gene expression in *max1-3* might be linked to the reduced flavonoid level in the stem, the expression of flavonoid and auxin transporters along the inflorescence stem was measured. There is an inverse correlation (Fig. 2*B*) between the expression pattern of auxin transporters (e.g., PIN1, PIN3, and PIN4) and flavonoid pathway genes. The most significant increase in auxin transporter mRNA levels was localized to the trunk and the lowermost stem segment of *max1-3*, where steady-state RNA levels of most flavonoid pathway genes were below the detection level.

There appears to be two modes of flavonoid gene regulation in the stem (Fig. 2*B*, wt lanes under flavonoid pathway genes). (*i*) In the upper stem, higher expression of the early genes *CHS* and *F3H* together with *FLS*, coupled with undetectable levels of *DFR* and *ANS*, probably reflects flavonol synthesis and no or reduced anthocyanin production. (*ii*) In the trunk, strong expression of *CHS*, *F3H*, and *DFR* and some *ANS* and *An2* but no *FLS* probably results in the synthesis of anthocyanins. The visible anthocyanin pigmentation in the wt and Txs lowermost stem segment (data not shown) is consistent with this conclusion. *MAX1* expression consistently increases the steady-state RNA levels of the flavonoid genes in the trunk, lower stem, and upper stem segments (Fig. 2*B*, compare lanes under flavonoid pathway genes for *max1* with those of wt and Txs). This expression pattern has significance for the mechanism(s) by which *MAX1* regulates flavonoid gene expression.

Vegetative Bud Outgrowth Correlates with Elevated Expression of Auxin Transporters in the Vegetative Stem and Bud. Auxin transporter gene expression in stem and buds of the dissected trunk was analyzed by RT-PCR (Fig. 3). Comparing the data for *max1-3* with wt revealed an organ-specific, ≈2 -fold increase in steady-state RNA in most transporters with the exception of PIN1 in the stem and PIN4 in the bud, which displayed an ≈2.7-fold and 4.5-fold increase, respectively. Expression of *AUX1*, *LAX1*, and *PIN8* increased primarily in the stem. Likewise, *PIN1*, *PIN3*, *PIN5*, and *PIN6* increased primarily in the stem but also increased in the bud. In contrast, *PIN4* showed an ≈1.6 -fold increase in the stem but an ≈4.5 -fold increase in the bud. These data suggest that elevated auxin transport in the stem and bud might be acting in concert to induce lateral outgrowth in *max1-3*. *PIN4*, which has the highest level of induction in the bud, could possibly play a distinct, bud-specific role(s).

Discussion

Our data demonstrate that *MAX1* is an essential positive regulator of the flavonoid pathway in the vegetative bud and underlying stem of *Arabidopsis*. Although *MAX1* is expressed in the stem from early germination, it does not become a limiting factor for flavonoid gene expression until the late vegetative stage, when axillary bud outgrowth is perceptible, indicating that *MAX1* control of flavonoid gene expression is temporally constrained. *MAX1* could regulate flavonoid gene expression either

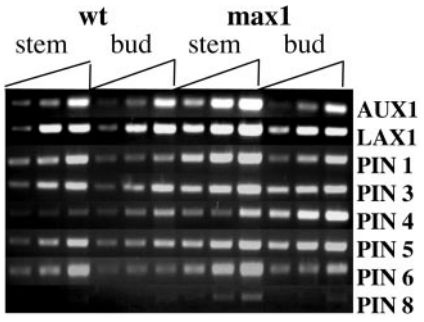

Fig. 3. Expression profiles of auxin transporter genes in the axillary bud and rosette stem of late-vegetative-stage plants. Semiquantitative RT-PCR was performed as described for Fig. 1*D*.

directly or by acting indirectly on *AN2/PAP1*, a transcriptional regulator of the flavonoid pathway (15).

Repression of bud outgrowth requires *MAX1*-dependent flavonoid gene expression. Previous results indicated that the flavonoid status of the plant plays a role in lateral outgrowth. Flavonoid gene overexpression represses outgrowth from axillary buds (15), whereas loss of flavonoids derepresses such outgrowth (12). That *MAX1*, a regulator of vegetative axillary bud outgrowth, is essential for flavonoid gene expression suggests that this flavonoid-based mechanism in late-vegetative-stage stems and buds is under the control of *MAX1*.

Evidence from *Arabidopsis* indicates that flavonoid deficiency leads to elevated transport of polar auxin (12). Our results support this finding. In *max1-3* there is an inverse correlation in expression between the flavonoid genes and the auxin transporter genes, strongly suggesting that the elevated auxin transport is a consequence of the reduced flavonoid level. The auxin transport profile in the wt *Arabidopsis* stem, lowest at the top and highest at the bottom (12), can also be explained by the differences in the flavonoid gene expression profile under the control of *MAX1* (see Fig. 2, where the majority of the changes occur in the trunk and lower stem). Furthermore, the flavonoid status alters expression of the *PIN* auxin efflux facilitator genes in *Arabidopsis* (13). Thus, flavonoid regulation of *PIN* genes at the steady-state RNA level appears to be a universal mechanism plants use to control auxin retention/movement.

Flavonoids are multifunctional effectors acting as inhibitors of protein phosphorylation (20) and topoisomerase (18), or, as signaling molecules (21), they can elicit a cascade of secondary events that may be relevant to *PIN* expression at multiple levels. Flavonoid-regulated vesicular cycling and targeting of PIN membrane transporters (13) as well as the inhibition of *MDR/PGP* transporters (22) may also be integrated into the auxin distribution mechanism of the bud and stem as well.

We propose here a working model for flavonoid repression of bud outgrowth. One element of the model is flavonoid repression of polar auxin transport in the stem due to *MAX1*-induced reduction of expression of auxin transporters. In the absence of *MAX1* function, the flavonoid level is reduced, leading to derepression of several auxin transporters and the subsequent increased auxin flow compromises apical dominance. This model is compatible with the idea that polar auxin flow in the vicinity of the axillary bud regulates bud outgrowth and that the degree of bud inhibition depends on the amounts of auxin in the polar transport stream in the stem (23).

The other and more central element of the model is that *MAX1*-dependent maintenance of a high-flavonoid state in the bud is essential for repression of bud outgrowth and that flavonoids repress *PIN4* and to a smaller extent a subset of auxin transporter genes in the bud (Fig. 3). Previous observations indicate that the lack of auxin export from the bud correlates with repression of bud outgrowth (24) and that there is a sharp decline in the bud auxin level at the time of bud release (25). A possible interpretation is that flavonoid accumulation could define bud repression at least in part via auxin retention. We speculate that flavonoid-dependent localization and distribution of auxin between the bud and stem probably is key for repression and that local changes in flavonoid level/distribution/composition in *max1-3* changes the auxin distribution/accumulation pattern and bud activity.

As vegetative buds are formed from single cells at the subepidermal layer (26) they are not connected *a priori* to the vascular system of the plant. Vascular development is known to require auxin signaling. In a classic experiment, Sachs (27) demonstrated that auxin induces differentiation of vascular strands that connect the site of auxin application with the preexisting vasculature. An attractive hypothesis is that *MAX1* may possibly control a flavonoid-gated auxin export mechanism from the bud to promote vascular development. High levels of flavonoids in the wt bud could specify auxin retention, as it does in other tissues (12, 28), and maintain the bud in the repressed state. In contrast, reduced levels of flavonoids could lead to auxin export from the bud that in turn drives development of vascular connections to the preexisting vasculature. Whether *MAX1* expression in the vascular bundles has any functional relevance in this process remains to be established. *PIN4*, the auxin efflux facilitator that showed significantly increased expression in the bud at the early stage of outgrowth, might have a role in this process.

Because flavonoid pathway regulation is subject to control by many environmental factors (such as nutrients, light, temperature, and water stress) that could affect lateral branching as well, flavonoids can uniquely satisfy requirements to mediate several environmental cues to bud repression.

Further experiments using double mutant analysis with mutations blocking key steps in the flavonoid pathway will be necessary to establish a causal effect of flavonoids on bud outgrowth and determine the extent to which the effects of *max1* might be independent of flavonoids.

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