

GRAMINIFOLIA promotes growth and polarity of *Antirrhinum* leaves

John F. Golz^{1,*}, Mario Roccaro^{1,†}, Robert Kuzoff^{2,‡} and Andrew Hudson^{1,§}

¹Institute of Cell and Molecular Biology, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR, UK

²Section of Plant Biology, University of California Davis, 1 Shields Avenue, Davis, CA 95616, USA

*Present address: Department of Biological Sciences, Monash University, Clayton, VIC 3800, Australia

†Present address: Max-Planck-Institut für Züchtungsforschung, Carl-von-Linné-Weg 10, 50829 Köln, Germany

‡Present address: Department of Plant Biology, The University of Georgia, Athens, GA 30602-7271, USA

§Author for correspondence (e-mail: andrew.hudson@ed.ac.uk)

Accepted 1 April 2004

Development 131, 3661–3670

Published by The Company of Biologists 2004

doi:10.1242/dev.01221

Summary

The leaves of higher plants develop distinct cell types along their adaxial-abaxial (dorsal-ventral) axes. Interaction between leaf primordium cells with adaxial and abaxial identities is necessary for lateral growth of the developing leaf blade. We show that the growth and asymmetry of leaves in *Antirrhinum majus* involves the related YABBY transcription factors GRAMINIFOLIA (GRAM) and PROLONGATA (PROL). GRAM is expressed in abaxial margins of organ primordia where it promotes lateral growth and abaxial cell fate. GRAM, however, is not needed for abaxial fate in the absence of adaxial cell specification, suggesting that it promotes abaxial fate by excluding adaxial identity. Although GRAM expression is abaxially

restricted, it functions redundantly with its abaxially expressed paralogue, PROL, and with the ubiquitously expressed PHANTASTICA gene to promote adaxial identity via intercellular signalling. This non cell-autonomous behaviour is consistent with the ability of GRAM in only the abaxial most cell layer to direct normal development of more adaxial cells. The contrasting roles of GRAM in promoting and inhibiting adaxial identity might serve to reinforce and maintain the distinction between adaxial and abaxial domains in the growing leaf primordium.

Key words: *Antirrhinum majus*, GRAMINIFOLIA, PROLONGATA, YABBY, Leaf asymmetry

Introduction

As a leaf primordium emerges from the periphery of the shoot apical meristem (SAM) it flattens perpendicular to its adaxial-abaxial axis and subsequently develops layers of functionally specialised cell types asymmetrically along this axis. The growth that flattens the organ occurs around the ad-abaxial boundary, is abolished in mutants that have lost either identity and occurs ectopically at novel boundaries (Eshed et al., 2001; Kerstetter et al., 2001; Kim et al., 2003; McConnell and Barton, 1998; Schichnes et al., 1997; Waites and Hudson, 1995). This suggests that a juxtaposition of cells with adaxial and abaxial identities in a developing leaf is required for lateral growth, presumably through cell-cell signalling. The same mechanism is likely to control ad-abaxial asymmetry and growth in other lateral organs, such as bracts and petals.

In *Arabidopsis*, the related HD-ZIP genes PHABULOSA (PHB), PHAVOLUTA (PHV) and REVOLUTA (REV) specify adaxial leaf identity. The activity of these genes normally becomes restricted to the adaxial domain of newly initiated leaf primordia (McConnell et al., 2001; Otsuga et al., 2001). The characterisation of gain-of-function PHB and PHV alleles, which result in their ectopic abaxial expression and adaxial fate, have suggested two mechanisms that might normally limit PHB and PHV activity to the adaxial domain of developing organs. One proposes that the HD-ZIP proteins are activated by binding a ligand adaxially, promoting their own expression so that gain-of-function mutations, which affect the potential ligand binding site, render the proteins constitutively active

(McConnell et al., 2001). The hypothetical ligand may come from the centre of the SAM, because leaf initials surgically isolated from the SAM fail to form adaxial cell types (Sussex, 1955). The second explanation is that a short microRNA (miRNA) complementary to wild-type RNA from the HD-ZIP loci causes degradation of PHB and PHV, and possibly REV, transcripts in the abaxial leaf domain (Emery et al., 2003; Reinhart et al., 2002; Rhoades et al., 2002). Consistent with this model is the finding that these miRNAs accumulate in the abaxial domain of *Arabidopsis* and maize lateral organs (Juarez et al., 2004; Kidner and Martienssen, 2004). Because transcripts from the gain-of-function alleles no longer match the miRNA perfectly and are resistant to degradation (Tang et al., 2003), they might persist in the abaxial domain to specify ectopic adaxial fate. This second model does not exclude the possibility that the HD-ZIP proteins are also activated by a ligand. However, these models make different assumptions about how organ asymmetry is first specified. Adaxial HD-ZIP activation by a ligand from the centre of the SAM could constitute the first step in organ polarisation, whereas inactivation by the abaxially localised miRNA implies that the organ is already polarised or that the miRNA is itself the polarising signal.

KANADI (KAN) genes, which are both necessary and sufficient for abaxial fate in *Arabidopsis* leaves (Eshed et al., 1999; Eshed et al., 2001; Kerstetter et al., 2001), are needed to limit HD-ZIP gene expression to an adaxial domain (Eshed et al., 2001). Because loss of PHB, PHV and REV activity has a

similar effect to ectopic adaxial expression of *KAN*, it has been suggested that *HD-ZIPs* act at least partly by restricting the domain in which *KAN* genes promote abaxial fate (Eshed et al., 2001; Emery et al., 2003). However, it is currently unclear how asymmetric expression of *HD-ZIP* and *KAN* genes is first established, and the extent to which each gene family acts by repressing the other.

Another family of transcription factor genes – the *YABBY* (*YAB*) genes – are implicated in abaxial organ fate because their expression is restricted abaxially in organ primordia and *yab* mutations disrupt development of abaxial cell types (Sawa et al., 1999; Siegfried et al., 1999; Villanueva et al., 1999). Reduced activity of the two *YAB* genes *FILAMENTOUS FLOWER* (*FIL*) and *YAB3* results in a partial loss of abaxial cell identity but not its replacement by adaxial identity, as in *kan* mutants (Eshed et al., 2001; Siegfried et al., 1999). Similarly, ectopic *YAB* expression is not sufficient to confer abaxial fate on all cells and the ability of ectopic *KAN* expression to abaxialise cells is not dependent on *FIL* or *YAB3* activity. The role of *YAB* genes in organ asymmetry is therefore enigmatic.

Although most flowering plants, in common with *Arabidopsis*, have asymmetric lateral organs the extent to which their regulatory mechanisms are conserved remains largely untested. In the distantly related eudicot, *Antirrhinum majus*, the *MYB* gene *PHANTASTICA* (*PHAN*) acts redundantly with other factors that are sensitive to cold to promote adaxial identity – loss of *PHAN* activity causes abaxialisation of organs and loss of lateral growth (Waites and Hudson, 1995; Waites et al., 1998). Reduced activity of the *Arabidopsis* *PHAN* orthologue, *ASYMMETRIC LEAVES1* (*AS1*), has a lesser effect on ad-abaxial organ asymmetry (Byrne et al., 2000; Xu et al., 2003) and it is unclear whether the different developmental requirements for *PHAN* and *AS1* reflect divergence in the functions of genes that they regulate. The only known target of *PHAN* – the homeobox gene, *HIRZINA*, which it represses in leaves and petals – does not cause asymmetry defects when mis-expressed (Golz et al., 2002). It is therefore not obvious whether *PHAN* might regulate the orthologues of genes that control organ asymmetry in *Arabidopsis*.

To further understand the control of organ asymmetry and growth we have analysed the roles of the paralogous *Antirrhinum* *YAB* genes, *GRAMINIFOLIA* (*GRAM*) and *PROLONGATA* (*PROL*). *GRAM* expression becomes confined to an abaxial domain at the margins of leaf primordia, where it promotes lateral growth and abaxial identity. However, the role of *GRAM* in promoting abaxial identity is redundant if adaxial fate is not specified, suggesting that *GRAM* acts to exclude adaxial identity – a role supported by ectopic abaxial expression of a *PHB* homologue in *gram* mutant leaves. Although expression of both *GRAM* and *PROL* is confined to an abaxial domain by *PHAN* activity, *GRAM* acts redundantly with *PHAN* and with *PROL* to promote adaxial organ identity non cell-autonomously. *GRAM* expression in only the abaxial epidermal cell layer of organ primordia is sufficient to confer normal identity and growth to more adaxial cells, indicating that *GRAM* promotes abaxial fate non cell-autonomously. The contrasting roles of *GRAM* in promotion and repression of adaxial fate might serve to define and reinforce an ad-abaxial boundary required for continued leaf growth.

Materials and methods

Plant material

The *gram*^{constans} (*gram-1*) and *gram*^{mutabilis} (*gram-2*) mutants (Baur, 1918) and the MAM265 line (Stubbe, 1966) came from the IPK, Gatersleben, Germany. The *gram-3* mutant arose in the transposon-active line JI.75 (Carpenter and Coen, 1990), and was a gift from Rosemary Carpenter and Enrico Coen (John Innes Centre, UK). It was shown to carry a single recessive mutation, *gram-3*, that failed to complement *gram-1* or *gram-2*.

Periclinal chimeras for an *olive* (*oli*) mutation, which prevents accumulation of chlorophyll at higher light intensities (Hudson et al., 1993), were generated from the unstable *oli-605* allele in *gram-1* and *GRAM*⁺ backgrounds. Plants were grown at 15°C for 10 days to induce excision of the Tam3 transposon from *oli-605*, then maintained at 25°C, to inhibit further transposition, in a light intensity of ~200 µmol/m²/second, to distinguish *OLI*⁺ revertant and *oli* mutant tissue. Chlorophyll was identified in hand-cut sections by epifluorescence at 365 nm excitation. Periclinal chimeras were maintained and propagated vegetatively from cuttings.

phan gram double mutants were obtained in the F₂ of either *phan-249* × *gram-1* (in the Sippe 50 genetic background), or *phan-607* × *gram-3* (in the JI.75 background). About 6% of F₂ progeny showed an enhanced mutant phenotype that included lack of an embryonic apical meristem. These plants were confirmed as *gram phan* double mutants by Southern hybridisation. *gram prol* and *phan prol* double mutants were obtained in the F₂ of *gram-1* × *prol-1* and *phan-249* × *prol-1*, respectively, and their genotypes confirmed by PCR.

Molecular biology

cDNA clones of *AmYAB2* (AY451398), *AmFIL* (*GRAM*, AY451396) and *AmYAB5* (*PROL*; AY451397) were obtained by low-stringency screening of an *Antirrhinum* inflorescence cDNA library with the *Arabidopsis* *INNER NO OUTER* gene (Villanueva et al., 1999). Additional cDNAs from these genes and two additional paralogues, *AmCRC* (AY451399) and *AmINO* (AY451400) were kindly provided by Zsuzsanna Schwarz-Sommer (MPIZ, Germany). The introns of *AMYAB3* and *AMYAB5* were identified by PCR amplification of genomic DNA. Sequence phylogenies were reconstructed from inferred full-length amino acid sequences using CLUSTAL and PAUP software.

Both *gram-1* and *gram-3* gave rise to a low frequency of wild-type progeny and *gram-3* produced wild-type branches, consistent with both mutations being caused by unstable transposons. Transposons were identified by PCR with transposon- and *GRAM*-specific primers. Primers to a sequence conserved in CACTA transposons were used with *AMYAB5*-specific primers to screen DNA from a collection of mutants maintained at IPK, Gatersleben. These detected a CACTA insertion within the first intron of *AmYAB5* in the inbred line, MAM265, which had slightly larger leaves than the wild-type lines, JI.75 and Sippe 50. In an F₂ of MAM265 × JI.75 (*n*=94) leaf size showed continuous variation and Student's *t*-tests detected no significant differences in leaf length or width between *amyab5/amyab5*, *amyab5/+* and homozygous wild-type siblings (*P*>0.20 in all pair-wise comparisons). This suggested that the *amyab5* allele did not condition a mutant phenotype and that the phenotype of MAM265 was consistent with a different genetic background to JI.75 or Sippe 50. The *amyab5* allele, however, segregated with an enhanced *gram* mutant phenotype in ~6% of the F₂ progeny of MAM265 × *gram-1*. These plants (*n*=23) were confirmed as *amyab5 gram-1* double mutants by PCR genotyping, while all 18 tested *gram* mutant siblings carried at least one wild-type *AmYAB5* allele, indicating enhancement of the *gram* phenotype by *amyab5*, or a very closely linked gene. Two *amyab5 gram* double mutants produced branches with a *gram* single mutant phenotype. PCR analysis confirmed that these branches carried revertant *AmYAB5* alleles with sequence footprints characteristic of CACTA transposon excision;

strongly suggesting that enhancement of the *gram* phenotype was due to the *amyab5* mutation. In the absence of other detectable mutations in MAM265, which had originally been proposed to carry the *prolongata* (*prol*) mutation, the *amyab5* allele was named *prol-1*.

Three *PHB* homologues, most similar to *PHB*, *REV* and *ATH-B8/ATH-15*, respectively, were obtained by probing an *Antirrhinum* cDNA library with a *PHB* cDNA. The most *PHB*-like gene (*AmPHB*; AY451395) encoded a protein with 84% identical amino acids to *PHB* in a 230 amino acid region spanning the START domain.

Microscopy

Epidermal impressions were made in Loctite Superglue on a microscope slide and examined with phase contrast optics. Histological sections (5 μ M) were made from material embedded in JB-4 resin and stained with Toluidine Blue (Ruzin, 1999). Scanning electron microscopy and in situ hybridisation were performed as described previously (Golz et al., 2002). The digoxigenin-labelled probes *GRAM*-long and *PROL* were transcribed from near full-length cDNA clones and the *GRAM* 3' probe from the final three exons downstream of the Tam3 insertion in *gram-3*. Antisense *AmPHB* probes were transcribed from a 700 bp cDNA that spanned the region encoding the START domain.

Results

GRAM is needed for adaxial-abaxial asymmetry and growth of lateral organs

The recessive *gram-3* mutation was identified in a transposon mutagenesis screen for altered leaf growth and shown to be allelic to two classic mutations, *gram-1* and *gram-2*. All three mutations had similar developmental effects.

Wild-type leaves differ in size according to the

node at which they are produced, reaching their maximum mature length and width at nodes 3 or 4 (Fig. 1A). All leaves of *gram-1* and *gram-2* mutants were consistently half the width of wild-type ones (Fig. 1A,B) and also shorter than wild type up to node 3, after which they were similar in length.

The adaxial epidermis of a wild-type *Antirrhinum* leaf consists of large irregular pavement cells with infrequent hairs and stomata (Fig. 1C), the abaxial epidermis comprises small pavement cells, fewer hairs but frequent stomata and cells at the leaf edge are domed and elongated. Internally, palisade mesophyll is found adaxial to spongy mesophyll and the junction between these tissues runs to the leaf edge (Fig. 1E). The lower chlorophyll content and larger air-spaces of the spongy mesophyll make the abaxial side of the leaf appear light green (Fig. 1B).

Leaves of *gram* mutants have regions of darker green tissue, characteristic of the adaxial side of the leaf, at the abaxial margins (Fig. 1B). In section, elongated palisade cells extend around the edge of the leaf into the abaxial margin (Fig. 1F), making the lamina thicker towards its edge. This phenotype

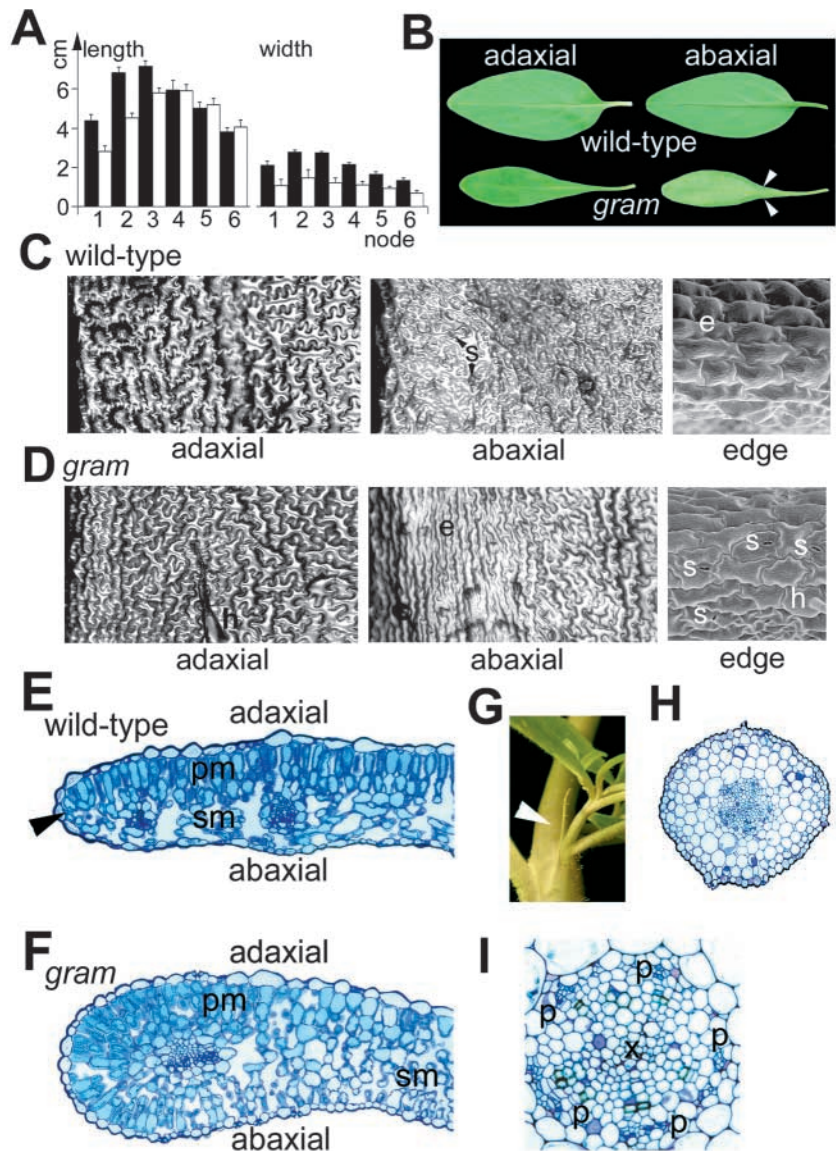


Fig. 1. *gram* mutations affect leaf growth and ad-abaxial asymmetry. (A) Comparisons of the length and width of fully expanded leaves of wild-type (filled bars) and *gram-1* (open bars) at nodes 1 to 6. Values represent means of eight replicates and error bars, one standard deviation. (B) The adaxial wild-type leaf (top left) appears darker than the abaxial (top right). In *gram* mutant leaves, strips of darker tissue extend abaxially (arrowheads). (C) The wild-type adaxial leaf surface (left) consists of large, irregular pavement cells and rectangular cells towards the leaf edge (left in this image). Abaxial pavement cells (centre) are smaller and interspersed with frequent stomata (s). The edge of the leaf (right) consists of elongated edge cells (e). (D) In *gram* mutants, adaxial pavement cells are unaltered whereas cells at the margins are more irregular (left), edge cells are found abaxially (centre) and larger abaxial cells are seen, and stomata and hairs (h) are found at the leaf edge. (E) A transverse section of a wild-type leaf shows that, the junction between adaxial palisade mesophyll (pm) and abaxial spongy mesophyll (sm) cells runs to the edge of the leaf (arrowhead). In the *gram* mutant leaf (F), palisade mesophyll cells (pm) are found abaxially at the margins and adaxial cells away from the margin resemble spongy mesophyll. (G,H) *gram* mutants occasionally produce needle-like leaves (arrowhead in G), that appear radial in transverse section (H). (I) The arrangement of xylem elements internal to phloem in central vascular bundles of these leaves suggests a loss of adaxial leaf identity.

suggested that *GRAM* is needed for abaxial cell identity at the leaf margin and that adaxial identity occurs in its absence. Similarly, epidermal cells with adaxial characters were found in the abaxial margins and the cells normally associated with the leaf edge extended further into the abaxial epidermis (Fig. 1D), suggesting that *GRAM* also promotes abaxial identity in epidermal cells. Epidermal marginal cells, which normally form at the leaf edge overlying the junction between spongy and palisade mesophyll, were absent from the displaced adaxial-abaxial boundary in *gram* leaves (Fig. 1B,D).

Loss of *GRAM* activity also caused adaxial mesophyll cells away from the leaf margin to partly resemble abaxial spongy mesophyll in shape and spacing (Fig. 1F). This suggested that *GRAM* is not only needed for abaxial identity at leaf margins but to promote adaxial identity elsewhere in the leaf. More severe loss of adaxial cell identity was observed occasionally in needle-like leaves produced by *gram-3* mutants (Fig. 1G), which contained a central vein in which xylem was surrounded by phloem (Fig. 1H,I). Because phloem develops abaxial to xylem in the wild-type leaf, the needle-like leaves appeared to have lost adaxial, and gained abaxial, identity. *gram* mutant petals, like leaves, were smaller than wild-type ones and free for more of their length (Fig. 2A,E), suggesting that *GRAM* is also needed for petal growth. Where petals remained united, pronounced furrows developed in their adaxial (inner) sides flanked by ridges (arrowheads in Fig. 2F,G). Cells within the furrow had ectopic abaxial identity, as seen by their darker red pigmentation and lack of yellow hairs. Similarly, the ridges flanking each furrow contained a radially symmetric vein with an abaxialised arrangement of cell types (compare Fig. 2B-D with Fig. 2F-H), suggesting that *GRAM* is needed for adaxial identity at petal margins. *gram* mutants also showed reduced growth of the style and occasional homeotic conversions of floral organ identity (Navarro et al., 2004).

***GRAM* promotes growth in a marginal domain of leaf primordia**

Although mature leaves of *gram* mutants were narrower than those of the wild type, they originated from primordia of similar size (data not shown), suggesting that the reduced width of *gram* leaves was a consequence of less growth after initiation. *gram* mutant leaves contained ~50% fewer cells in the lateral axis when compared to a wild-type leaf at the same node (Fig. 1), suggesting that reduced cell divisions were involved in the reduced leaf width. To test whether reduced cell division occurred throughout the developing leaf, or whether it was largely confined to a particular region, we analysed the contributions of different cell layers to wild-type and *gram* mutant leaves.

In *Antirrhinum*, in common with most dicots, the SAM consists of three cell populations – a single layer of protoderm cells (L1), a single layer of sub-epidermal cells (L2) and a core of L3 cells. The fates of cells derived from the L2 layer were followed in *GRAM*⁺ and *gram* mutant leaves using stable periclinal chimeras in which L2 was marked by an *olive* mutation that reduces chlorophyll content (see Materials and methods). Towards the midrib of *GRAM*⁺ leaves, L2 contributed one layer of yellow adaxial palisade cells and one abaxial layer of yellow spongy mesophyll cells covering a core of L3-derived green cells (Fig. 3A). The medial part of the leaf therefore appeared green. Nearer the leaf edges, all internal

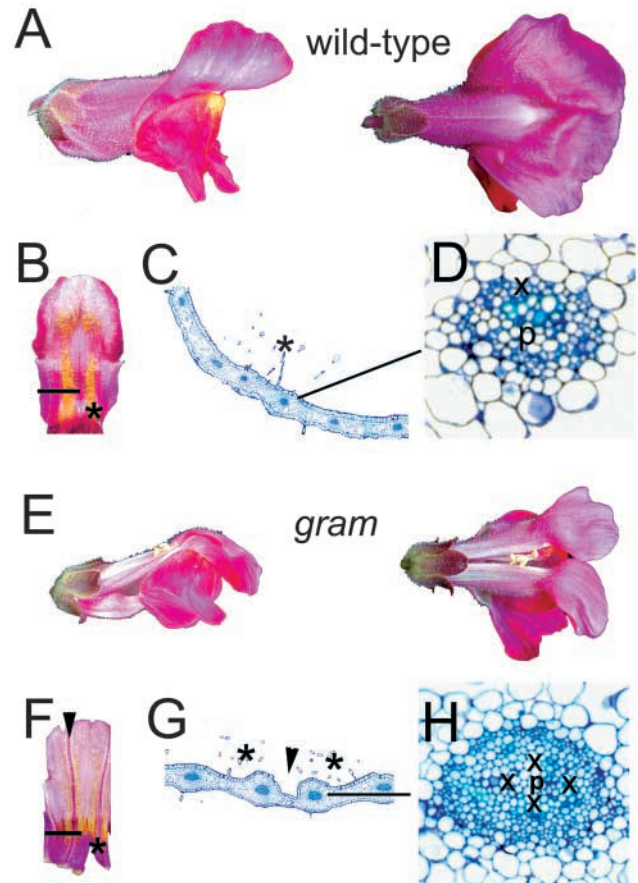


Fig. 2. *gram* mutations affect growth and asymmetry of petals. (A-D) A wild-type flower. (A) A lateral (left) and dorsal (right) view. (B) Two rows of dense yellow hairs (*), are present on the adaxial surface at the junction between the ventral and lateral petals within the corolla tube. (C) In a section, taken at the position of the bar in B, the junction between petals is flanked by veins in which xylem elements (x) are adaxial to phloem (p; D). (E-H) *gram* mutant flower. (E) Mutant flowers are smaller than wild-type ones and the dorsal petals are free laterally and more symmetrical in shape. (F) The junction between ventral and lateral petals (arrowhead in F) is flanked by ridges containing enlarged abaxialised veins (G, and enlarged in H) that have central xylem elements (x) surrounded by phloem (p). Epidermal tissue between the ridges (arrowhead in G) has abaxial identity, as evidenced by a higher level of anthocyanin pigmentation characteristic of abaxial petal epidermis and absence of yellow hairs.

cells were derived from L2 and therefore the margins appeared yellow. The proportion of the blade with internal L2-derived cells varied from about one-third to two-thirds of the leaf width (Fig. 3A). The boundary between green (L3-derived) and yellow (L2-derived) tissue did not correspond to any structural feature and its position varied in different leaves or in opposite halves of the same leaf. In contrast, L3 contributed most of the internal cells in a *gram* mutant leaf (often more cells than in *GRAM*⁺) and the position of the boundary between yellow and green tissue was more consistent (Fig. 3B). This suggested firstly, that *GRAM* promotes cell divisions in the margins of leaf primordia, where internal tissues are derived entirely from L2, and secondly that L3 contributes more cells to the *gram*

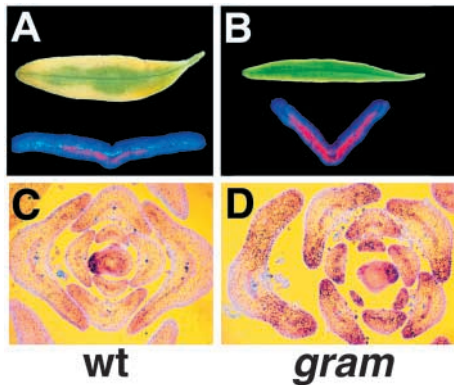


Fig. 3. *GRAM* promotes marginal leaf growth. (A) The leaves of periclinal chimeras in which L2-derived cells carry an *oli* mutation that reduces chlorophyll content. L2-derived cells contribute a variable proportion of the internal cells of the leaf – seen as a yellow marginal region in the surface view (above) or as cells showing no red chlorophyll auto-fluorescence under UV light in section (below). (B) The loss of L2-derived tissues at the leaf margins of *gram* mutants is partially compensated for by an increased growth of the L3 layer. (C,D) Transverse sections of wild-type (C) and *gram* (D) vegetative apices probed with the *CYCLIND3a* probe. Note the shift in *CYCLIN* expression to more internal regions of *gram* mutant leaves.

mutant leaf than to the wild-type leaf, perhaps in compensation for reduced marginal growth.

To test when in development *GRAM* activity was required for localised cell proliferation, the expression of *CYCLIN D3a* RNA, which correlates with cell division rates in organ primordia (Gaudin et al., 2000), was compared in *gram* and wild-type leaves. Expression in newly initiated wild-type primordia was uniform and then became concentrated in the growing margins, remaining detectable there until at least stage P5 (Fig. 3C). *gram* mutant primordia resembled those of wild type in size early in development and showed a similar early pattern of *CYCLIN D3a* expression (Fig. 3D). However, from stage P3 or P4, expression spread from the margins to more central cells and persisted there until at least stage P7, consistent with a shift in growth from a marginal to more central region of the leaf later in development.

GRAM encodes YABBY activity

Both *gram-1* and *gram-3* showed the genetic instability characteristic of transposon-induced mutations. Because *GRAM* was needed to promote abaxial organ identity and a similar role had been attributed to members of the *YAB* gene family in *Arabidopsis* (Siegfried et al., 1999), we tested whether *GRAM* might encode YAB activity. Five *Antirrhinum* *YAB* genes were identified as cDNAs, each encoding a protein with the N-terminal zinc finger and C-terminal HMG-like YAB domain characteristic of the family (Fig. 4B).

Phylogenetic analysis placed one of these proteins, termed AmFIL, in a well-supported clade with the products of the *Arabidopsis* *FILAMENTOUS FLOWER* (*FIL*) and *YAB3* genes (Fig. 4A). A second *Antirrhinum* protein, AmYAB5, appeared orthologous to *YAB5*. Amplification from genomic DNA identified six introns in *AmFIL* and *AmYAB5* in positions that

were conserved between *Antirrhinum* genes and with their *Arabidopsis* homologues (Fig. 4B). An *AmFIL* probe detected a different RFLP in each *gram* mutant that segregated with the *gram* mutant allele (data not shown). Sequence analysis revealed that the RFLPs were caused by transposon insertions; *gram-3* carried a copy of the Tam3 transposon in exon 5 of *AmFIL* and *gram-1* and *gram-2* carried Tam2 in introns 4 or 5, respectively (Fig. 4B). The *gram-3* allele had the potential to encode a protein in which the C-terminal part of the highly conserved YAB domain was replaced with 69 amino acids encoded by Tam3.

Tam3 was lost from *gram-3* in five independent *GRAM*⁺ revertants. None carried sequence footprints, often associated with Tam3 excision, presumably because footprints would disrupt the highly conserved YAB domain. A single reversion of *gram-1* to wild-type involved the loss of Tam2 together with 85 bp of flanking intron sequence. These results confirmed that *AmFIL* corresponded to the *GRAM* locus.

A transposon insertion in the *AmYAB5* gene was also identified in an inbred line carrying the classic mutation, *prolongata-1* (*prol-1*; Fig. 4B) (Stubbe, 1966), but conditioned no mutant phenotype in an otherwise wild-type genetic background (see Materials and methods).

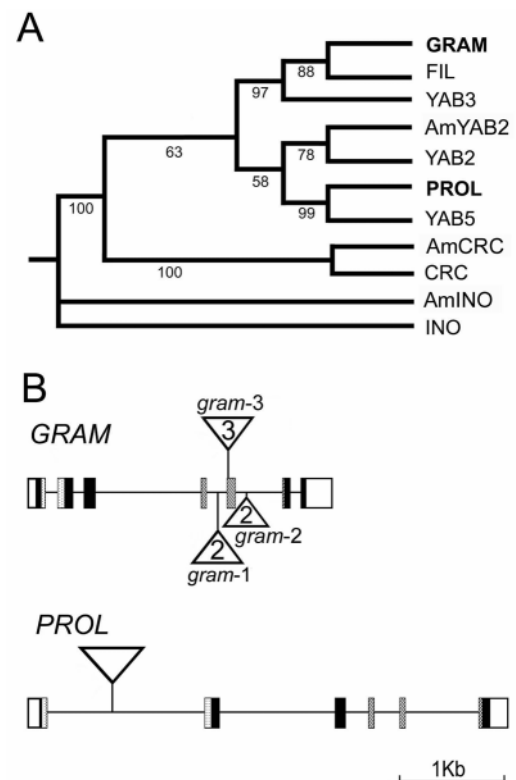


Fig. 4. Structure and evolution of *GRAM* and *PROL*. (A) A neighbour-joining tree showing the relative similarity of the full-length *Antirrhinum* and *Arabidopsis* YABBY proteins, suggesting their evolutionary relationships. Bootstrap values (1000 replicates) are given. (B) The structure of the *GRAM* and *PROL* loci and mutant alleles. Boxes represent exons (black are translated, white are un-translated). The regions encoding the N-terminal zinc finger domain and the C-terminal YAB domain are stippled. Transposon insertions are shown as triangles (not to scale); numbers denote Tam2 or Tam3.

***GRAM* and *PROL* are expressed abaxially in developing lateral organs**

GRAM RNA expression, revealed by in situ hybridisation, was similar in all lateral organs. It was detected first in incipient primordia within the SAM or floral meristem (stage P0 in leaves) and abaxially in newly initiated (early P1) primordia (Fig. 5A,B). It then became restricted mainly to the abaxial margins of growing primordia from about stage P2 (Fig. 5B). This later pattern of expression was consistent with the proposed role of *GRAM* in promoting abaxial cell fate and growth in leaf margins. *PROL* RNA was always less abundant than *GRAM* (Fig. 5D,E), but like *GRAM* it was expressed abaxially from stage P1. Later expression, unlike *GRAM*, was detected predominantly in provascular cells and to a lesser extent in the mesophyll cells in the centre of each primordium. *PROL* RNA was not detectable in the *prol-1* mutant by in situ hybridisation or RT-PCR (data not shown).

***GRAM*, *PROL* and *PHAN* promote adaxial organ fate**

GRAM and *PROL* RNA, which are expressed in abaxial cells

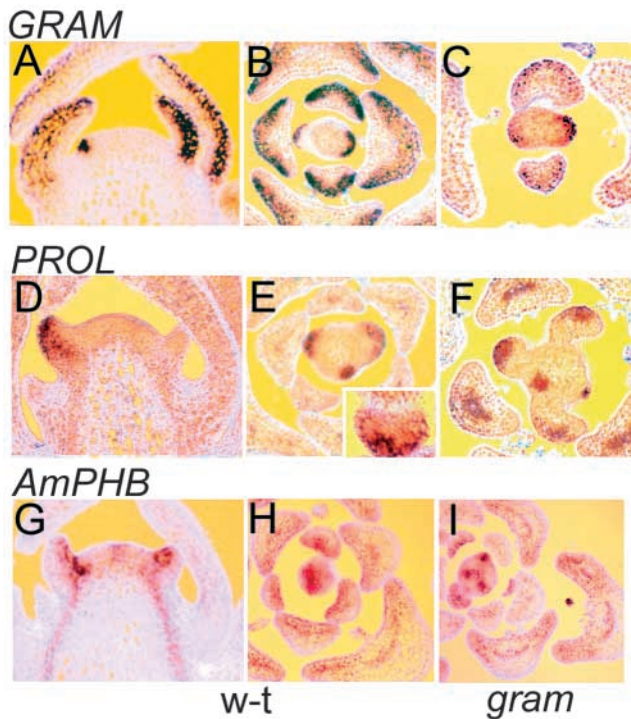


Fig. 5. Expression of *GRAM*, *PROL* and *AmPHB* RNA. In situ hybridisation to detect *GRAM* (A–C), *PROL* (D–F) and *AmPHB* (G–I) RNA. (A,D,G) Longitudinal sections and (B,E,H) transverse sections of wild-type apices; (C,F,I) transverse sections of *gram* mutant apices. The youngest leaf primordia are towards the centre of each apex. (A) *GRAM* transcript (detected as a dark precipitate) is abaxially restricted and becomes most abundant in the abaxial margins (B). *PROL* expression in wild type (D,E) is abaxial in newly initiated primordia (inset in E) and later confined to the developing midveins of leaves. (G,H) Expression of *AmPHB* RNA is detected within the wild-type SAM and throughout leaf initials within it, becomes restricted to an adaxial region of leaf primordia after initiation then to adaxial leaf margins and to the vasculature of leaves and stem. In *gram* mutant leaves, *GRAM* (C) and *PROL* (F) expression is lost from the marginal, abaxial domain of developing leaves, whereas *AmPHB* now extends into this region (I).

of wild-type leaf primordia, were absent from the margins of *gram* mutant primordia (Fig. 5C,F), consistent with the loss of abaxial identity from this region. *GRAM* expression was unaffected by the *prol-1* mutation that has no effect on leaf development (data not shown).

Loss of PHANTASTICA (*PHAN*) activity has the opposite effect to *gram* of allowing leaf cells in adaxial positions to assume abaxial fates (Waites and Hudson, 1995). The degree to which *phan* mutant leaves are abaxialised increases with decreasing temperature: at 25°C all leaves are mosaics of adaxial and ectopic abaxial tissue (Fig. 6C), at 20°C leaves at higher nodes on the plant are needle-like and consist only of abaxial cell types (Fig. 6A) while at 15°C *phan* mutants are unable to maintain a functional SAM.

Because *GRAM* is required for abaxial identity, ectopic abaxial fate in *phan* mutant leaves might result from ectopic *GRAM* expression. Consistent with this, the domain of *GRAM* expression was found to extend into the adaxial region of *phan* mutant primordia initiated at 20°C (Fig. 6I,J). If *GRAM* activity was responsible for ectopic abaxial identity, *gram* mutations would be expected to suppress the polarity defects of *phan* mutant leaves. However, *gram* mutations were found to enhance, rather than suppress the *phan* mutant phenotype. *phan gram* double mutant seedlings had more severely abaxialised cotyledons and differentiated cells in place of the SAM (Fig. 6E–H). Shoots eventually arose from adventitious meristems in the hypocotyl or the base of cotyledon petioles (Fig. 6B,D). Leaves produced from *phan gram* shoots were radially symmetrical and abaxialised, based on their histology, arrangement of vascular cells in the central vein (Fig. 6K,L) and ubiquitous expression of mutant *gram* RNA (Fig. 6M). Loss of *GRAM* activity also rendered *phan* mutants insensitive to temperature (Fig. 6A–D). Enhancement of the *phan* mutant phenotype by *gram* mutations suggested that *GRAM* promotes adaxial organ fate redundantly with *PHAN*. Because the leaves of *phan gram* double mutants retain abaxial identity, *GRAM* also appeared unnecessary for abaxial organ identity in the absence of adaxial fate specification.

To determine whether *GRAM* and *PROL* function redundantly to promote abaxial identity, plants were generated carrying both *gram* and *prol-1* mutations. Surprisingly *prol-1* enhanced the *gram* mutant phenotype in the same way as *phan* mutations (Fig. 6N–S). Initially all *gram prol* seedlings lacked a SAM, however shoots eventually formed from adventitious meristems at the root-hypocotyl junction (Fig. 6N). All of the leaves that formed on these shoots were radially symmetrical and had hairs that were specific to the abaxial surface of wild-type leaves (Fig. 6O–Q). Lack of adaxial cell types was confirmed by histology (Fig. 6R) and by the ubiquitous expression of *gram* mutant transcript and complete absence of *AmPHB* expression (Fig. 6S,T). These results suggest that *PROL* promotes adaxial organ fate redundantly with *GRAM* and that it is not required, alone or redundantly with *GRAM*, for abaxial fate when adaxial fate is not specified. Unlike *gram* mutations, *prol-1* did not modify the *phan* mutant phenotype (data not shown).

Neither *GRAM* nor *PROL* are needed for abaxial cell fate in the absence of adaxial identity. The role of *GRAM* in promoting abaxial organ fate might therefore be to repress adaxial identity. To test this we examined its interaction with *AmPHB*, an *Antirrhinum* homologue of *PHB*, which is necessary and

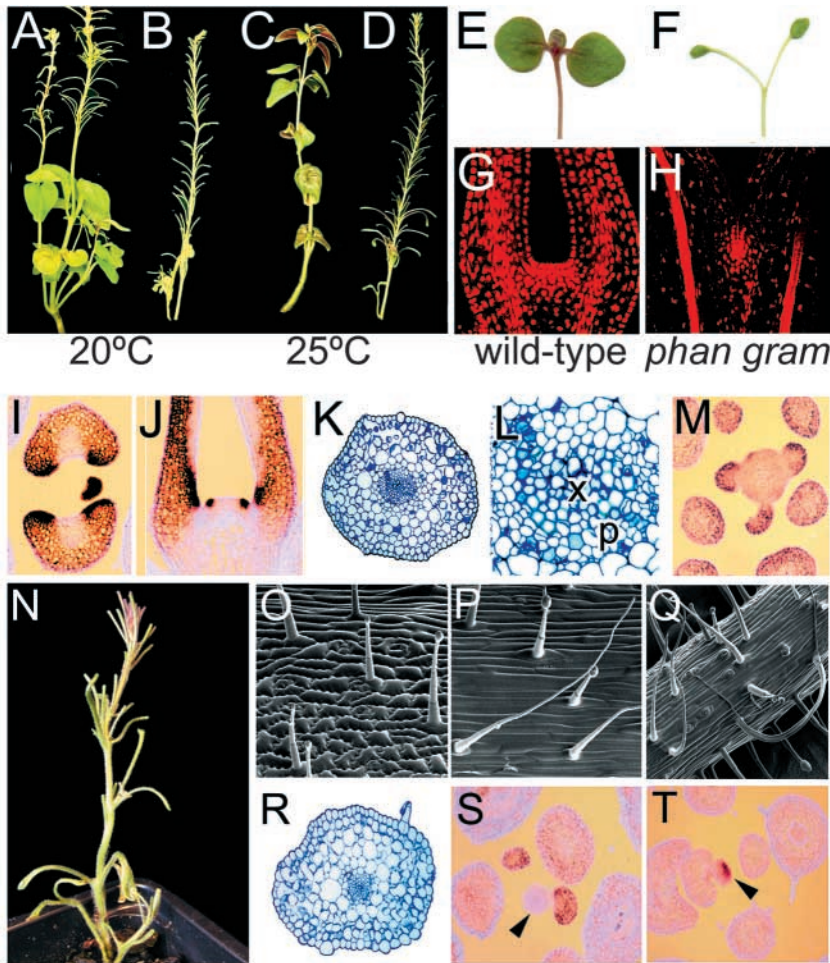


Fig. 6. Interactions between *gram*, *phan* and *prol* mutations. *phan* single mutants (A,C) and *phan gram* double mutants (B,D) grown at either 20°C or 25°C. *gram* enhances the abaxialised phenotype of *phan* leaves and removes their sensitivity to temperature. Wild type, *gram* and *phan* single mutants produce a functional embryonic SAM, seen to have a layered structure in an optical section of a newly germinated seedling (G), that gives rise to a shoot between the cotyledons (E). *phan gram* double mutants have abaxialised cotyledons (F) and fail to form an organised or functional SAM during embryogenesis (H). The apices of *phan* mutants grown at 20°C, seen in transverse section (I) and longitudinal section (J) express *GRAM* RNA ectopically in adaxial regions of developing leaves. The needle-like leaves of *phan gram* double mutants are radially symmetrical in transverse section (K), have an abaxialised arrangement of xylem internal to phloem (L) and express *gram* RNA ubiquitously (M). (N) *prol gram* double mutants also fail to form an embryonic SAM and produce radially symmetrical leaves (Q) that have the long glandular hairs characteristic of the wild-type abaxial (P) rather than adaxial (O) midrib; show abaxialisation of internal cell types (R); ubiquitous expression of *gram* RNA (S) and reduced expression of *AmPHB* RNA (T), although *AmPHB* expression remains in the SAM (arrowhead).

sufficient for adaxial fate in *Arabidopsis* leaves (McConnell et al., 2001). Sense RNA from *AmPHB*, like its *Arabidopsis* homologue, was expressed in the wild-type SAM, uniformly in newly initiated leaf primordia and adaxially from late stage P1 (Fig. 5G,H). In contrast, *AmPHB* expression was not adaxially restricted in P2 and P3 primordia of *gram* mutants and was particularly abundant at their margins (Fig. 5I). This expression pattern was therefore consistent with *GRAM* acting to repress *AmPHB* expression and adaxial identity from at least stage P2.

GRAM acts non cell-autonomously

GRAM and *PROL* are expressed abaxially but required, non cell-autonomously, to promote the identity of adaxial cells. To test whether *GRAM* can also affect abaxial fate non cell-autonomously, we exploited the ability of the *gram-3* mutation to give rise to clones of wild-type cells following transposon excision. Plants homozygous for the unstable *gram-3* allele occasionally produced branches with a wild-type phenotype. In most cases, the flowers on these branches gave rise to ~75% wild-type progeny on self-pollination, suggesting that the subepidermal (L2) layer of the SAM, from which gametes are derived, carried a revertant *GRAM*⁺ allele. One phenotypically wild-type branch, however, produced only *gram* mutant progeny, suggesting that it was a periclinal chimera carrying a revertant *GRAM*⁺ allele in either the L1 or L3 layers of the

SAM. These possibilities were tested by in situ hybridisation with a probe that could detect wild-type *GRAM* RNA but not the transcripts produced from *gram-3*, which terminate within the transposon insertion (Fig. 7A-D). In the chimeric wild-type branch, the downstream probe detected high levels of *GRAM* transcripts only in L1 cells within the normal, abaxial domain of *GRAM* expression (Fig. 7F), indicating that this branch had normal *GRAM* activity in epidermal cells but not in sub-epidermal, L2-derived cells. Consistent with this, a wild-type *GRAM*⁺ allele could be amplified from the revertant branch, but not from *gram* mutant branches of the same plant. These findings indicated that *GRAM* activity in abaxial epidermal cells is sufficient for normal identity and proliferation of more adaxial cells, presumably via an intercellular signal.

Discussion

We have shown that the YABBY transcription factor, *GRAM*, is needed for abaxial identity and growth at the margins of *Antirrhinum* leaves. This role correlates with *GRAM* expression in an abaxial domain of newly initiated organ primordia and its persistence in abaxial margins of the growing leaf.

Loss of *GRAM* activity allows cells in abaxial marginal positions to assume adaxial identities. Because the leaves of

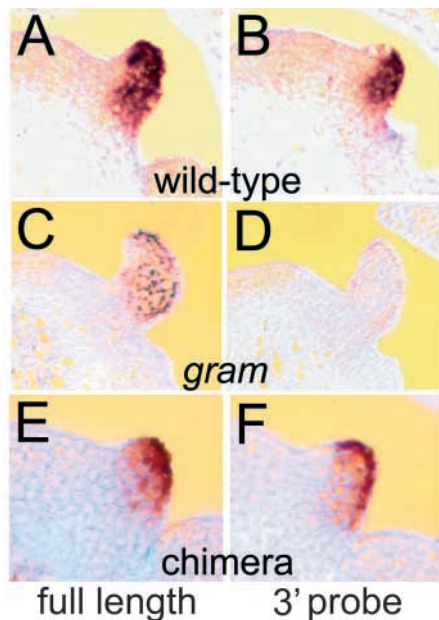


Fig. 7. *GRAM* RNA expression in a periclinal chimera. In situ hybridisation with either a full-length *GRAM* cDNA probe (A,C,E) or a transcribed region of *GRAM* downstream from the transposon insertion in *gram-3* (3' probe; B,D,F). Both probes detect wild-type *GRAM* transcripts (A,B). Transcripts from *gram-3* are detected, at a reduced level, by the full-length probe (C) but not the 3' probe (D). In the phenotypically wild-type shoot of a periclinal chimera, the full-length probe detects *GRAM* transcripts in all cell layers of developing leaves (E) whereas the 3' probe detects a high level of transcripts only in the epidermal cell layer (F), indicating that the shoot carries a *GRAM*⁺ revertant allele in the epidermal, L1 cell layer. Each row shows adjacent sections from the same shoot apex and the sections in each column were probed together on the same microscope slide.

plants lacking *GRAM* activity in a *phan* or *prol* mutant background retain abaxial identity, *GRAM* does not appear to be necessary for abaxial fate per se, but rather to exclude adaxial identity from the abaxial leaf margins. This does not exclude the possibility that other genes might specify abaxial identity independently of *GRAM*. Obvious candidates include other members of the *YAB* gene family, at least two of which – *PROL* and *AmYAB2* – are expressed abaxially in developing leaves of wild-type and abaxialised *phan gram* mutants (data not shown). *PROL*, however, is not needed for abaxial identity, alone or in combination with *GRAM*, because abaxial identity is retained in leaves lacking activity of both *GRAM* and *PROL* or *PHAN* and *PROL*. In *Arabidopsis*, members of the *KAN* family are also required for abaxial fate (Eshed et al., 2001; Kerstetter et al., 2001). Two *KAN* genes are known to be expressed in *Antirrhinum* leaves (J.F.G., unpublished) and might therefore specify abaxial fates in the absence of *GRAM* and *PROL* activity.

Activity of the HD-ZIP proteins PHB, PHV and REV is sufficient to confer adaxial identity in *Arabidopsis* leaves (Emery et al., 2003; McConnell et al., 2001) and restriction of their activity to an adaxial domain is considered to be an early step in elaboration of organ asymmetry. In *Antirrhinum* leaves, *GRAM* is needed to restrict expression of *AmPHB*, a *PHB*

homologue, to an adaxial domain of organ primordia, consistent with *GRAM* acting to repress HD-ZIP-dependent adaxial fate. It is, however, unclear whether *GRAM* is needed to set up the domain of *HD-ZIP* expression or to maintain it. It is also unclear whether abaxial expression of *GRAM* is established in response to adaxial HD-ZIP activity.

The role of *GRAM* in repressing adaxial fate differs from that proposed for the homologous *Arabidopsis* genes, *FIL* and *YAB3*. Reduced activity of both *Arabidopsis* genes has a similar effect to *gram* mutations on leaf growth (Kumaran et al., 2002; Siegfried et al., 1999), suggesting that *FIL* and *YAB3* together provide a *GRAM*-like function. However, *fil yab3* mutants have less severe polarity defects involving only a partial loss of abaxial cell characters but no clear gain of adaxial identity. In this respect the effects of *gram* mutations are more similar to loss of both *KAN1* and *KAN2* activity in *Arabidopsis*, which is also accompanied by ectopic *HD-ZIP* expression, as in *gram* (Eshed et al., 2001). The different requirements for *GRAM* compared to *FIL* and *YAB3* might reflect divergence in the function of the *YAB* gene family in the two species (e.g. from *GRAM* having assumed or retained *KAN*-like functions). Alternatively, they might result from different degrees of functional overlap between *YAB* genes within each species. These possibilities might be tested with additional *yab* loss-of-function mutations in both species. In the case of *Antirrhinum*, reduced activity of *PROL*, for which no orthologous *Arabidopsis* mutant has been reported, has no developmental effects when *GRAM* is active, suggesting that *PROL* is redundant. Because reduced activity of both *GRAM* and *PROL* results in loss of adaxial identity, it does not reveal whether *PROL* and *GRAM* might function redundantly to repress adaxial identity.

In addition to promoting abaxial fate by repression of adaxial identity, *GRAM* and *PROL* together promote adaxial identity. This role is apparent in the loss of adaxial cell characters from *gram* single mutant leaves and the complete replacement of adaxial by abaxial tissues in *gram prol* double mutant leaves. In *gram prol*, the polarity defect is also accompanied by reduced SAM activity, as seen in other mutants with abaxialised leaves (e.g. Eshed et al., 2001; Waites and Hudson, 2001). Although *GRAM* is expressed ectopically in the abaxialised leaves of *phan* mutants, *gram* mutations also enhance the abaxialised organ phenotype of *phan* mutants in a similar way to the *handlebars* (*hb*) mutation (Waites and Hudson, 2001) and, like *hb*, remove its sensitivity to cold. This is consistent with *HB* and *GRAM* acting in a cold-sensitive pathway that promotes adaxial identity redundantly with *PHAN*. However, the relationship between these genes is likely to be more complex because *hb gram* double mutants (not shown) resemble *hb phan*, *gram phan* and *gram prol* mutants, whereas the *prol* mutation enhances the phenotype of *gram*, but not *phan* mutants.

The finding that the *KNOX* gene, *HIRZ*, is expressed ectopically in abaxialised leaves of *phan* mutants has led to the suggestion that *KNOX* expression might cause polarity defects (e.g. Tsianstis et al., 1999). In *Arabidopsis*, *FIL* and *YAB3* have also been found necessary to prevent *KNOX* expression in leaves (Kumaran et al., 2002), suggesting that *GRAM* might have a similar role in *KNOX* repression and that the enhanced mutant phenotype of the *phan gram* mutant might reflect increased *KNOX* mis-expression. Two observations,

however, argue against this. Firstly, ectopic *KNOX* expression could not be detected in *gram* single mutant leaves and secondly, a *Hirz* gain-of-function mutation that causes ectopic *HIRZ* expression, as in *phan* mutants, failed to cause leaf polarity defects in a *GRAM*⁺ background or to enhance the polarity defects of *gram* mutants (data not shown) (Golz et al., 2002).

Because both *GRAM* and *PROL* show abaxially restricted expression but promote adaxial identity, they appear to be necessary for a non cell-autonomous signal from the abaxial to adaxial domain. A further non cell-autonomous role of *GRAM* was revealed by a periclinal chimera in which *GRAM* expression only in the most abaxial cell layer (L1) of the primordium was sufficient for normal development of leaves and flowers. The simplest explanation for both these non cell-autonomous effects is that they involve the same intercellular signalling mechanism. Because *GRAM* protein is absent from the adaxial region of developing leaves (Navarro et al., 2004), it is likely to regulate production of a downstream signal in abaxial cells, rather than to act as a signal itself.

Loss of adaxial identity is also observed in plants lacking activity of *GRAM* and *STYLOSA* (*STY*) (Navarro et al., 2004), suggesting that *STY* is also required for the adaxial promoting signal. *GRAM* and *STY* proteins interact physically and are co-expressed only in abaxial cells of early organ primordia, suggesting that *STY* and *GRAM* together regulate the signalling mechanism from early in organ development.

GRAM has opposite roles in the two parts of the leaf – repression of adaxial identity in the abaxial domain and promotion of adaxial identity. This seems unlikely to result from differences in the concentration of a signalling molecule, because the boundary of *GRAM* expression can be shifted abaxially to the junction between L1-L2 cell layers in a periclinal chimera without causing a shift in the boundary between development of adaxial and abaxial tissues.

Although the adaxial-promoting and adaxial-repressing roles of *GRAM* might appear paradoxical, similar phenomena appear to be common in other signal-response systems. For example, the Decapentaplegic signalling protein is secreted by the most dorsal cells of the *Drosophila* embryo and promotes expression of the *Zerknüllt* (*Zen*) transcription factor, which confers amniosera fate, in a dorsal domain (Ray et al., 1991). It also induces more ventral expression of *Brinker* (*Br*), which represses *Zen* transcription cell-autonomously (Jazwinska et al., 1999). The interaction of *Br* with *Zen* is necessary to refine the dorsoventral boundary of *Zen* expression (Muller et al., 2003). In an analogous way the opposite effects of *GRAM* might serve to refine the boundary between adaxial and abaxial cells of organ primordia preventing the specification of intermediate cell identities. It might also serve to maintain the boundary, which is proposed to be necessary for lateral growth. This view of *GRAM* function is consistent with the observed loss of ad-abaxial distinction at the margins of *gram* leaves and the loss of lateral growth in this region. Use of *CYCLIN D3a* expression as a marker for cell division, suggested that lateral growth of primordia was not affected until relatively late in development, consistent with a requirement for *GRAM* to maintain an ad-abaxial boundary. Both adaxial asymmetry and growth are maintained in the medial parts of the leaf, perhaps because of the activity of other genes (e.g. additional *YAB* family members). A requirement for *GRAM* to maintain a

growth-promoting ad-abaxial boundary is also consistent with the lack of ectopic growth at the ectopic boundary between adaxial and abaxial cell types in the ventral margin of *gram* mutant leaves.

Evidence for ab-adaxial signalling in leaves has also been provided by *Arabidopsis* plants with reduced activity of the abaxially expressed *KANI* gene. *kan1* mutants show abaxial defects, but also dosage-dependent reductions in adaxial trichome density (Kerstetter et al., 2001).

We are grateful to Tracy Dransfield and Gianna Romano for genotyping plants, Melissa Spielman and Sandra Floyd for advice on histological analysis and Zsuzsanna Schwarz-Sommer for discussion and providing EST sequences. We would also like to thank three anonymous reviewers for their comments and suggestions. This work was supported by the Biotechnology and Biological Sciences Research Council and a European Molecular Biology Organisation Fellowship to J.F.G.

References

- Baur, E. (1918). Mutationen von *Antirrhinum majus*. Z. f. indukt. Abst. Vererbungsl. **19**, 177-193.
- Byrne, M. E., Barley, R., Curtis, M., Arroyo, J. M., Dunham, M., Hudson, A. and Martienssen, R. A. (2000). *Asymmetric leaves1* mediates leaf patterning and stem cell function in *Arabidopsis*. *Nature* **408**, 967-971.
- Carpenter, R. and Coen, E. S. (1990). Floral homeotic mutations produced by transposon mutagenesis in *Antirrhinum majus*. *Genes Dev.* **4**, 1483-1493.
- Emery, J. F., Floyd, S. K., Alvarez, J., Eshed, Y., Hawker, N. P., Izhaki, A., Baum, S. F. and Bowman, J. L. (2003). Radial patterning of *Arabidopsis* shoots by class III HD-ZIP and KANADI genes. *Curr. Biol.* **13**, 1768-1774.
- Eshed, Y., Baum, S. F. and Bowman, J. L. (1999). Distinct mechanisms promote polarity establishment in carpels of *Arabidopsis*. *Cell* **99**, 199-209.
- Eshed, Y., Baum, S. F., Perea, J. V. and Bowman, J. L. (2001). Establishment of polarity in lateral organs of plants. *Curr. Biol.* **11**, 1251-1260.
- Gaudin, V., Lunness, P. A., Fobert, P. R., Towers, M., Riou-Khamlichi, C., Murray, J. A., Coen, E. and Doonan, J. H. (2000). The expression of *D-cyclin* genes defines distinct developmental zones in snapdragon apical meristems and is locally regulated by the *Cycloidea* gene. *Plant Physiol.* **122**, 1137-1148.
- Golz, J. F., Keck, E. J. and Hudson, A. (2002). Spontaneous mutations in *KNOX* genes give rise to a novel floral structure in *Antirrhinum*. *Curr. Biol.* **12**, 515-522.
- Hudson, A., Carpenter, R., Doyle, S. and Coen, E. S. (1993). *Olive*: a key gene required for chlorophyll biosynthesis in *Antirrhinum majus*. *EMBO J.* **12**, 3711-3719.
- Jazwinska, A., Rushlow, C. and Roth, S. (1999). The role of *brinker* in mediating the graded response to *Dpp* in early *Drosophila* embryos. *Development* **126**, 3323-3334.
- Juarez, M. T., Kui, J. S., Thomas, J., Heller, B. A. and Timmermans, M. C. P. (2004). MicroRNA-mediated repression of *rolled leaf1* specifies maize leaf polarity. *Nature* **428**, 84-88.
- Kerstetter, R. A., Bollman, K., Taylor, R. A., Bomblies, K. and Poethig, R. S. (2001). *KANADI* regulates organ polarity in *Arabidopsis*. *Nature* **411**, 706-709.
- Kim, M., Pham, T., Hamidi, A., McCormick, S., Kuzoff, R. K. and Sinha, N. (2003). Reduced leaf complexity in tomato *wiry* mutants suggests a role for *PHAN* and *KNOX* genes in generating compound leaves. *Development* **130**, 4405-4415.
- Kidner, C. A. and Martienssen, R. A. (2004). Spatially restricted microRNA directs leaf polarity through ARGONAUTE1. *Nature* **428**, 81-84.
- Kumaran, M. K., Bowman, J. L. and Sundaresan, V. (2002). *YABBY* polarity genes mediate the repression of *KNOX* homeobox genes in *Arabidopsis*. *Plant Cell* **14**, 2761-2770.
- McConnell, J. R. and Barton, M. K. (1998). Leaf polarity and meristem formation in *Arabidopsis*. *Development* **125**, 2935-2942.
- McConnell, J. R., Emery, J., Eshed, Y., Bao, N., Bowman, J. and Barton, M. K. (2001). Role of *PHABULOSA* and *PHAVOLUTA* in determining radial patterning in shoots. *Nature* **411**, 709-713.
- Muller, B., Hartmann, B., Pyrowolakis, G., Affolter, M. and Basler, K.

- (2003). Conversion of an extracellular Dpp/BMP morphogen gradient into an inverse transcriptional gradient. *Cell* **113**, 221-233.
- Nakajima, K., Sena, G., Nawy, T. and Benfey, P. N.** (2001). Intercellular movement of the putative transcription factor SHR in root patterning. *Nature* **413**, 307-311.
- Navarro, C., Efremova, N., Golz, J. F., Rubiera, R., Kuckenberg, M., Castillo, R., Tietz, O., Saedler, H. and Schwarz-Sommer, Z.** (2004). Molecular and genetic interactions between *STYLOSA* and *GRAMINIFOLIA* in the control of *Antirrhinum* vegetative and reproductive development. *Development* **131**, 3649-3659.
- Otsuga, D., DeGuzman, B., Prigge, M. J., Drews, G. N. and Clark, S. E.** (2001). *REVOLUTA* regulates meristem initiation at lateral positions. *Plant J.* **25**, 223-236.
- Perbal, M., Haughn, G., Saedler, H. and Schwarz-Sommer, Z.** (1996). Non cell-autonomous function of the *Antirrhinum* floral homeotic proteins DEFICIENS and GLOBOSA is exerted by their polar cell- to-cell trafficking. *Development* **122**, 3433-3441.
- Ray, R., Arora, K., Nusslein-Volhard, C. and Gelbart, W.** (1991). The control of cell fate along the dorsal-ventral axis of the *Drosophila* embryo. *Development* **113**, 35-54.
- Reinhart, B. J., Weinstein, E. G., Rhoades, M. W., Bartel, B. and Bartel, D. P.** (2002). MicroRNAs in plants. *Genes Dev.* **16**, 1616-1626.
- Rhoades, M. W., Reinhart, B. J., Lim, L. P., Burge, C. B., Bartel, B. and Bartel, D. P.** (2002). Prediction of plant microRNA targets. *Cell* **110**, 513-520.
- Ruzin, S. E.** (1999). *Plant Microtechnique and Microscopy*. New York: Oxford University Press.
- Sawa, S., Watanabe, K., Goto, K., Kanaya, E., Morita, E. H. and Okada, K.** (1999). *FILAMENTOUS FLOWER*, a meristem and organ identity gene of *Arabidopsis*, encodes a protein with a zinc finger and HMG-related domains. *Genes Dev.* **13**, 1079-1088.
- Schichnes, D., Schneeberger, R. and Freeling, M.** (1997). Induction of leaves directly from leaves in the maize mutant *Lax midrib1-O*. *Dev. Biol.* **186**, 36-45.
- Sessions, A., Yanofsky, M. F. and Weigel, D.** (2000). Cell-cell signaling and movement by the floral transcription factors LEAFY and APETALA1. *Science* **289**, 779-781.
- Siegfried, K. R., Eshed, Y., Baum, S. F., Otsuga, D., Drews, G. N. and Bowman, J. L.** (1999). Members of the *YABBY* gene family specify abaxial cell fate in *Arabidopsis*. *Development* **126**, 4117-4128.
- Stubbe, H.** (1966). *Genetik und Zytologie von Antirrhinum L. sect. Antirrhinum*. Jena: Gustav Fischer Verlag.
- Sussex, I. M.** (1955). Morphogenesis in *Solanum tuberosum* L.: Experimental investigation of leaf dorsoventrality and orientation in the juvenile shoot. *Phytomorphology* **5**, 286-300.
- Tang, G., Reinhart, B. J., Bartel, D. P. and Zamore, P. D.** (2003). A biochemical framework for RNA silencing in plants. *Genes Dev.* **17**, 49-63.
- Tsiantis, M., Schneeberger, R., Golz, J. F., Freeling, M. and Langdale, J. A.** (1999). The maize *rough sheath2* gene and leaf development programs in monocot and dicot plants. *Science* **284**, 154-156.
- Villanueva, J. M., Broadhvest, J., Hauser, B. A., Meister, R. J., Schneitz, K. and Gasser, C. S.** (1999). *INNER NO OUTER* regulates abaxial-adaxial patterning in *Arabidopsis* ovules. *Genes Dev.* **13**, 3160-3169.
- Waites, R. and Hudson, A.** (1995). *phantastica*: a gene required for dorsoventrality in leaves of *Antirrhinum majus*. *Development* **121**, 2143-2154.
- Waites, R. and Hudson, A.** (2001). The *Handlebars* gene is required with *Phantastica* for dorsoventral asymmetry of organs and for stem cell activity in *Antirrhinum*. *Development* **128**, 1923-1931.
- Waites, R., Selvadurai, H. R., Oliver, I. R. and Hudson, A.** (1998). The *PHANTASTICA* gene encodes a MYB transcription factor involved in growth and dorsoventrality of lateral organs in *Antirrhinum*. *Cell* **93**, 779-789.
- Xu, L., Xu, Y., Dong, A., Sun, Y., Pi, L. and Huang, H.** (2003). Novel *as1* and *as2* defects in leaf adaxial-abaxial polarity reveal the requirement for *ASYMMETRIC LEAVES1* and 2 and *ERECTA* functions in specifying leaf adaxial identity. *Development* **130**, 4097-4107.