AMP1 and MP antagonistically regulate embryo and meristem development in Arabidopsis

Danielle P. Vidaurre, Sara Ploense*, Naden T. Krogan and Thomas Berleth†

AUXIN RESPONSE FACTOR (ARF)-mediated signaling conveys positional information during embryonic and postembryonic organogenesis and mutations in MONOPTEROS (MP/ARF5) result in severe patterning defects during embryonic and postembryonic development. Here we show that MP patterning activity is largely dispensable when the presumptive carboxypeptidase ALTERED MERISTEM PROGRAM 1 (AMP1) is not functional, indicating that MP is primarily necessary to counteract AMP1 activity. Closer inspection of the single and double mutant phenotypes reveals antagonistic influences of both genes on meristematic activities throughout the Arabidopsis life cycle. In the absence of MP activity, cells in apical meristems and along the paths of procambium formation acquire differentiated identities and this is largely dependent on differentiation-promoting AMP1 activity. Positions of antagonistic interaction between MP and AMP1 coincide with MP expression domains within the larger AMP1 expression domain. These observations suggest a model in which auxin-derived positional information through MP2005b; Riou-Khamlichi et al., 1999). However, the AMP1 phenotype. Despite a wealth of phenotypic data, AMP1 function has not been genetically linked to other genes in embryo or meristem patterning. The AMP1 product bears similarities to mammalian N-acetyl α-linked acidic dipeptidases (NAALADases) (Helliwell et al., 2001), but neither its organismal or cellular localization nor the molecular identity of its targets is known.

Auxin distribution patterns have been implicated in positioning of lateral organs in shoots and roots (Reinhardt et al., 2003; Benkova et al., 2003), the formation of vascular tissues (Aloni et al., 2003; Avsian-Kretchmer et al., 2002; Mattsson et al., 2003) and in the generation of the root stem cell niche (Aida et al., 2004). In all these positions, robust patterns of auxin accumulation were found associated with patterned cell fate specification, including positioning of meristematic activities. Other plant hormones, specifically cytokinins, are also essential for promoting cell division (Bishopp et al., 2006), but their distribution patterns have not been as precisely correlated to cellular responses. Auxin regulates gene expression through auxin response factors (ARFs) and their nuclear co-regulators of the Aux/IAA family (Guilfoyle and Hagen, 2001; Liscum and Reed, 2002). Although most ARF functions are still elusive, patterning functions involving organ initiation and growth have been assigned to some ARFs, including MONOPTEROS (MP/ARF5). Mutations in MP lead to the absence of an embryonic root, the formation of reduced vascular systems and flowerless shoots (Berleth and Jürgens, 1993; Przemeck et al., 1996). Among other ARFs, ARF7/NON-PHOTOTROPIC HYPOCOTYL 4 and ARF19 are required for local cell proliferation in the pericycle to produce lateral roots (Okushima et al., 2005a; Wilmoth et al., 2005). By contrast, ARF2 has been shown to restrict the size of Arabidopsis ovules and seeds and to negatively regulate certain cell proliferation genes (Ellis et al., 2005; Okushima et al., 2005b; Schuff et al., 2005).

Here we identify amp1 as a first loss-of-function suppressor of an arf mutant and present evidence that AMP1 has a role in balancing and restricting the meristem-promoting activity of auxin signaling. We document that MP has an important role in promoting meristematic niches in diverse locations and that this activity is dispensable in the absence of a counteracting pathway involving AMP1.

INTRODUCTION

Cell division is unequally distributed in the plant body. Growth regions with controlled patterns of dividing cells, termed apical meristems, elongate the plant axis and an extended meristem, the procambium, retains pluripotent cells for subsequent vascular differentiation. In many locations, proliferative activity is inversely correlated with the differentiation status of cells and their balance defines the sizes of meristematic regions. In some meristematic tissues, as for example in the procambium, dividing cells give rise to limited cell numbers in specialized tissues (Esau, 1965), whereas in apical meristems permanent stem cells give off daughter cells indefinitely (Weigel and Jürgens, 2002).

The controls regulating the balance between proliferating and differentiating cells are only partially understood. Where amenable to genetic dissection, as in the shoot apical meristem (SAM), these controls seem to comprise antagonistic activities acting in specific zones (reviewed by Bäurle and Laux, 2003; Williams and Fletcher, 2005). Antagonistic activities might also control the size of other meristems. A mechanism related to that in the SAM has been proposed for the root meristem (Casamitjana-Martinez et al., 2003), and the formation of procambium in the leaf seems to occur in competition with mesophyll differentiation (Scarpella et al., 2004).

Mutations in the presumptive glutamate carboxypeptidase AMP1 are associated with diverse morphological abnormalities including supernumerary cotyledons, shortened plastochrons and a bushy appearance, and are further characterized by cytokinin overproduction and upregulation of CYCD3;1 (Chaudhury et al., 1993; Chin-Atkins et al., 1996; Nogué et al., 2000a; Nogué et al., 2000b; Riou-Khamlichi et al., 1999). However, amp1 mutants are phenotypically distinct from both cytokinin or CYCD3;1-overproducing plants and it is unclear what primary defect could account for the various aspects of the amp1 phenotype. Despite a wealth of phenotypic data, AMP1 function has not been genetically linked to other genes in embryo or meristem patterning. The AMP1 product bears similarities to mammalian N-acetyl α-linked acidic dipeptidases (NAALADases) (Helliwell et al., 2001), but neither its organismal or cellular localization nor the molecular identity of its targets is known.

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Here we identify amp1 as a first loss-of-function suppressor of an arf mutant and present evidence that AMP1 has a role in balancing and restricting the meristem-promoting activity of auxin signaling. We document that MP has an important role in promoting meristematic niches in diverse locations and that this activity is dispensable in the absence of a counteracting pathway involving AMP1.

KEY WORDS: amp1, Arabidopsis, Embryogenesis, Meristem, mp, Stem cells

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MATERIALS AND METHODS

Plant material and growth conditions

Unless otherwise noted, seeds were plated and plants grown as previously described (Hardtke et al., 2004). Origin of transgenic lines: CycB1;1::CycB1;1-GUS (Donnelly et al., 1999), pCLV3::GUS (Brand et al., 2002), SNO-GFP (Cutler et al., 2000). UBI3::Li6b-GFP was generated by M. Aida in the laboratory of B. Scheres (Utrecht University, Utrecht, The Netherlands) by fusion of membrane marker 29-1 (Cutler et al., 2000) to the potato UBI3 promoter (L22576).

Microtechniques and microscopy

Cleared whole-mount samples were prepared as described in Berleth and Jürgens (Berleth and Jürgens, 1993). Detection of β-glucuronidase (GUS) activity was as in Scarpella et al. (Scarpella et al., 2004) with the following modifications to the concentrations of potassium ferro- and ferricyanide and incubation times: 10 mM for 1 hour (pCLV3::GUS), 0.5 mM for 16 hours (MP::MP-GUS embryos), 2 mM for 2 hours (CycB1;1::CycB1;1-GUS), 5 mM for 2 hours (MP::MP-GUS seedlings) or 5 mM plus 1% Triton X-100 for 1 hour (MP::MP-GUS nuclear localization). Scanning electron microscopy and confocal laser scanning microscopy were performed as described (Douglas et al., 2002; Gazzarrini et al., 2004).

Sizes of inflorescence meristems were determined on images taken from above the meristem by measuring the distance from the centre of the youngest recognizable floral primordium to the centre of the crown separating the fifth flower primordium from the meristem as described (Yu et al., 2000). SAM sizes were determined on cleared medium longitudinal images using ImageJ 1.33 software (http://rsb.info.nih.gov/ij/) as being the area formed by the dome of the meristem connected by a straight line between the cotyledon primordia.

RESULTS AND DISCUSSION

A survey of the molecular lesions and phenotypic strengths of amp1 mutations, including six new alleles, identified amp1-1 and amp1-13 as likely null alleles with no recognizable AMP1 transcripts and amp1-1 as an allele with pronounced residual gene activity (see Fig. S1 and Table S1 in the supplementary material). As there are no apparent AMP1 paralogs in the Arabidopsis genome, the two allele-strength categories probably reflect partial and complete loss of NAALADase activity in the AMP1 pathway.

AMP1 function stabilizes suspensor cell fate and restricts cell numbers in embryos

Cell numbers and cell division patterns in the early wild-type Arabidopsis embryo are almost invariable. A particularly reproducible feature of the Arabidopsis embryonic fate map is the restriction of the descendants of the apical and basal daughter cells of the zygote. Basal cell descendants form the suspensor, but, except for the central portion of the root meristem, they do not contribute to the seedling pattern (Scheres et al., 1994). In amp1 embryos, abnormal divisions of basal cell derivatives gave rise to additional cell tiers in the embryo proper and basal cell derivatives regularly contributed to large parts of the seedling, including the hypocotyl and cotyledons (Fig. 1.I,I-P-S; frequency of extra tiers in amp1-13: 36/42, 42/54, 63/63, 96/96 at 4-, 8-, 16-cell and globular stages, respectively). Conversely, cells from the apical part of the globular embryo (framed cells in Fig. 1.G,H) no longer contributed to the seedling but became incorporated into an oversized SAM (Fig. 1.Q,R,S). At lower frequency, abnormal divisions of basal cell derivatives led to the formation of a complete second embryo from the same zygote, which was reflected in the appearance of twin seedlings from single seeds in amp1 mutant lines (Fig. 1.V and see Table S1B in the supplementary material). Except for the oversized shoot meristem and frequent supernumerary cotyledons, the architecture of amp1 late-stage embryos is remarkably normal (Fig. 1.Y) (Conway and Poethig, 1997). These features suggest that the mutant phenotype is primarily a consequence of the increased cell numbers in early pro-embryos.

In conclusion, the patterning defects in amp1 mutant embryos can be traced back to the failure of basal cell descendants to attain suspensor cell fate. Instead of displaying suspensor-specific differentiation features, some of those cells proliferate and either generate additional embryos or contribute to inappropriately large portions of the embryo proper.

AMP1 negatively regulates meristematic activities in shoots and roots

The enlarged SAM is not solely a consequence of abnormal cell specification in the embryo. As shown in Fig. 2.B,F, the pCLV3::GUS expression domain was five times wider than in wild type and became concentrated in concrete spots (Fig. 2.M). These spots might be correlated with the formation of multiple SAMs (Fig. 2.N), which we observed in all amp1 alleles, reminiscent of what has been described for the corona mutant (Green et al., 2005). By contrast, the sizes of the amp1 inflorescence and floral meristems were not markedly abnormal [mean inflorescence meristem diameter±s.e.m.: wild type, 52.6±0.7 mm (n=16); amp1-9, 51.4±1.2 mm (n=12)] (Fig. 2.W).

In hypocotyls and roots, pericycle cells retain proliferation competence, but only a portion of them will later progress through the cell cycle to produce lateral roots (Beeckman et al., 2001; Himanen et al., 2002). In amp1 mutants, the proportion of pericycle cells that were actually proliferating was greatly increased. Lateral roots were initiated very early (Fig. 2.A,A; Table 1C) and adventitious roots from hypocotyls were frequent (Fig. 2.A,E; Table 1B). Once established, amp1 mutant root meristems were not expanded in diameter (data not shown).

In summary, our results document that AMP1 restricts stem cell pool sizes in the SAM and keeps division-competent cells dormant in the pericycle.

MP promotes meristem formation in roots and shoots

Mutations in mp are associated with the absence of an embryonic root (Berleth and Jürgens, 1993) and MP has been implicated in the generation of a stem cell niche in the root meristem (Aida et al., 2004). As shown in Fig. 2.D,H,L, MP was found to promote stem cell formation not only in the root meristem, but also in the SAM. In wild type, the SAM is initiated during embryogenesis and produces the first two leaf primordia approximately at the time of germination (Laux et al., 1996). In mp mutants, the SAM was typically not visible at germination and SAMs were also smaller in mp seedlings (Fig. 2.D,H,L; Table 1A). As previously reported, mutant inflorescence meristems are unable to produce normal numbers of flowers, floral meristems produce fewer floral organs (Fig. 2.U,Y) (Przemeck et al., 1996) and mp mutants have incomplete vascular systems (Fig. 3.A). This defect has been traced back to a reduced procambium, the meristematic precursor tissue of vascular strands (Przemeck et al., 1996). Finally, we found that mp mutants produce adventitious roots only after many weeks in culture, in sharp contrast to the enhanced production of adventitious roots in amp1 mutants (Table 1B). In summary, mp mutants are defective in the generation of appropriately sized meristems in various locations.
amp1 uncouples embryo and meristem development from MP dependence

We assessed the possibility that MP and AMP1 activity antagonize each other in the control of meristematic activities by constructing amp1 mp double mutants of various allelic combinations. We found that amp1 suppresses the phenotype of mp and can even restore viability and fertility in an mp mutant background. Whereas rootless mp mutant seedlings were not viable under normal growth conditions, amp1 mp double mutants frequently formed hypocotyls and roots. Abnormal suspensor cell divisions (F) lead to a massive three-dimensional cell arrangement (I), clearly recognizable as part of the embryo proper by the triangular stage (L,Q).

Abnormal suspensor cell divisions (F) lead to a massive three-dimensional cell arrangement (I), clearly recognizable as part of the embryo proper by the triangular stage (L,Q). Note the presence of a basally extended epidermal layer (I) and the presence of additional cell tiers in the embryo proper in amp1 mutants (numbered in I). Note the emergence of cotyledon primordia from basal positions and of an oversized SAM at triangular (P), heart (Q-S) and torpedo (T) stage in amp1 mutants. Quantification of cell tier numbers (as illustrated in I) for wild-type and amp1 globular-stage embryos (n values between 42 and 51; error bars indicate s.e.m.).

Formation of a second embryo (outlined cells) and of twin-seedlings from single seeds in amp1-13 mutant seeds. Arrows in W point to two separate roots. Bent-cotyledon-stage embryos of wild-type (X), amp1-9 (Y), amp1-9 mpG92 (Z) and mpG92 (AA) genotype. The embryo in Z represents a largely normalized individual from a spectrum of embryonic phenotypes. All images except W are cleared whole-mounts viewed with DIC optics. ut, upper tier; lt, lower tier. Scale bars: 20 μm in A-J,V; 50 μm in K-T,X-AA.

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We assessed the possibility that MP and AMP1 activity antagonize each other in the control of meristematic activities by constructing amp1 mp double mutants of various allelic combinations. We found that amp1 suppresses the phenotype of mp and can even restore viability and fertility in an mp mutant background. Whereas rootless mp mutant seedlings were not viable under normal growth conditions, amp1 mp double mutants frequently formed hypocotyls and roots (Fig. 1Z, Fig. 2AB, Table 1D) and could be grown on soil (Fig. 2O-Q). In fact, amp1 mp double-mutant embryo development could be indistinguishable from amp1 embryogenesis (data not shown). Further, in contrast to the generally flower-defective and invariably sterile mp mutants, inflorescences of amp1 mp double mutants had abundant fertile flowers with partially restored numbers of floral organs (Fig. 2T,X, Table 1E). Mutations in AMP1 also increased the reduced cotyledon numbers in mp mutants (Table 1F) and we observed a restoration of adventitious root formation in amp1 mp double mutants (Table 1B). Finally, loss of AMP1 function significantly restored vascular tissue formation in the mp mutant background (Fig. 3A). Whereas the mature vascular system in cotyledons of mp mutants was typically restricted to a short midvein and occasional short side branches, cotyledon venation of amp1 mp double mutants comprised several lateral circular veins similar to wild-type cotyledons.

Suppression of mp by amp1 mutations was observed in a variety of allelic combinations. The resulting phenotypes support the notion of quantitative antagonistic activities, because the suppression of mp was generally weaker in combinations involving the weak amp1-1 allele (Fig. 2P,Q, Table 1D). In summary, our results indicate that MP and AMP1 genetically interact in the regulation of meristematic activity.
Fig. 2. Postembryonic interaction of AMP1 and MP in Arabidopsis. (A-D) Scanning electron micrographs showing relative sizes of SAMs of indicated genotypes at 3 days after germination (DAG). (E-L) Cleared whole-mount preparations viewed with DIC optics. Expression of stem cell marker pCLV3::GUS in the SAM of the indicated genotypes at 3 DAG (E-H) and 5 DAG (I-L). Note that expression of pCLV3::GUS extends beyond the most-central domain in the amp1 background. Numerical values of shoot meristem sizes are shown in Table 1A. (M) Discrete domains of pCLV3::GUS expression (arrowheads) in amp1 are associated with the formation of multiple meristems. (N) Formation of multiple SAMs (arrowheads) on expanded apices in amp1 mutants, which are clearly not associated with leaf axils. (O) Left to right: wild type, amp1-9, amp1-9 mpG92 and mpG92. Note that amp1 mp double mutants are smaller than amp1 mutants and have restored flower formation, which is defective in mp mutants. (P, Q) Gene dosage-specific phenotypes of amp1 mp double mutants. Note that the restoration of flower formation is far more complete in a double mutant comprising a strong amp1 and a weak mp allele (amp1-9 mpG92 in P) than in combination with a strong mp allele (amp1-1 mpBS1354 in Q). (R-U) Flower phenotypes. Normal flower organs in amp1 mutants (amp1-9 in S), inflorescence with few, highly reduced flowers in mpG92 mutants (U) and intermediate, fertile flowers in amp1-9 mpG92 mutants (T). (V-Y) Scanning electron micrographs of inflorescences indicating that no size abnormalities were observed in amp1 mutants. (Z-AC) Light-grown seedlings at 7 DAG. Note the advanced stage of the lateral root system in amp1 mutants. (AD-AG) Dark-grown seedlings at 14 DAG. Adventitious roots formed in amp1-9 and amp1-9 mpG92 mutants (arrows point to hypocotyl-root junction). Scale bars: 100 μm in A-D, V-Y; 50 μm in E-M; 1 mm in R-T, 5 mm in Z-AC; 2 mm in N, AD-AG.
Table 1. Developmental features of wild-type and *amp1*, *mp* and *amp1 mp* mutant plants

### A. Shoot meristem size

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Ler</th>
<th><em>amp1</em>-9</th>
<th><em>amp1</em>-9 <em>mpG92</em></th>
<th><em>mpG92</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Meristem area, μm²</td>
<td>356±28 (32)</td>
<td>5548±551 (25)*</td>
<td>5220±593 (29)*</td>
<td>26±2 (49)*</td>
</tr>
<tr>
<td>Meristem width, μm</td>
<td>47±2 (32)</td>
<td>148±6 (25)*</td>
<td>152±7 (29)*</td>
<td>14±3 (49)*</td>
</tr>
<tr>
<td>Meristem height, μm</td>
<td>11±1 (32)</td>
<td>54±4 (25)*</td>
<td>52±4 (29)*</td>
<td>3±1 (49)*</td>
</tr>
</tbody>
</table>

### B. Meristem size, 3 DAG

| Meristem area, μm²            | 589±77 (28)       | 9214±988 (18)*    | 8766±902 (23)*    | 211±23 (26)*    |
| Meristem width, μm             | 62±3 (28)         | 196±7 (18)*       | 190±9 (23)*       | 40±3 (26)*      |
| Meristem height, μm            | 14±1 (28)         | 70±6 (18)*        | 68±6 (23)*        | 8±2 (26)*       |

* Values are mean±s.e.m., (n). Note that only (13/49) and (10/26) *mpG92* mutants produced shoot apical meristems (SAMs) at 3 days after germination (DAG) and 5 DAG, respectively. Mutants that failed to produce a SAM were included in the calculations and given a measurement value of zero since all mutants will form SAMs subsequently.

### B. Frequency of adventitious root formation

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Ler</th>
<th><em>amp1</em>-9</th>
<th><em>amp1</em>-9 <em>mpG92</em></th>
<th><em>mpG92</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. adv roots, 14 DAG, dark</td>
<td>0.2±0.1 (55)</td>
<td>3.4±0.2 (51)†</td>
<td>1.5±0.1 (46)†</td>
<td>0 (39)†</td>
</tr>
<tr>
<td>No. adv roots, 7 DAG, light</td>
<td>1.0±0.6 (21)</td>
<td>2.2±1.1 (26)†</td>
<td>1.2±0.7 (45)</td>
<td>0 (36)†</td>
</tr>
</tbody>
</table>

* Values are mean number of adventitious roots from the hypocotyl±s.e.m., (n).

### C. Early emergence of lateral roots is observed in *amp1* seedlings at 4 DAG

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Number of initiation sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>39</td>
<td>0.1±0.05</td>
</tr>
<tr>
<td><em>amp1</em>-9</td>
<td>37</td>
<td>3.0±0.21†</td>
</tr>
<tr>
<td><em>amp1</em>-9 <em>mpG92</em></td>
<td>37</td>
<td>0.5±0.12†</td>
</tr>
<tr>
<td><em>mpG92</em></td>
<td>40</td>
<td>0±0</td>
</tr>
</tbody>
</table>

### D. Restoration of seedling pattern (rooted seedlings with hypocotyls at 10 DAG)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of rooting individuals</th>
<th>Total (n)</th>
<th>Frequency of rooting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>amp1</em>-1 <em>mpBS1354</em></td>
<td>35</td>
<td>338</td>
<td>10.3</td>
</tr>
<tr>
<td><em>amp1</em>-1 <em>mpG12</em></td>
<td>8</td>
<td>195</td>
<td>4.1</td>
</tr>
<tr>
<td><em>amp1</em>-1 <em>mpG33</em></td>
<td>12</td>
<td>136</td>
<td>8.8</td>
</tr>
<tr>
<td><em>amp1</em>-11 <em>mpBS1354</em></td>
<td>57</td>
<td>147</td>
<td>38.8</td>
</tr>
<tr>
<td><em>amp1</em>-10 <em>mpG12</em></td>
<td>40</td>
<td>132</td>
<td>30.3</td>
</tr>
<tr>
<td><em>amp1</em>-8 <em>mpG92</em></td>
<td>64</td>
<td>197</td>
<td>32.5</td>
</tr>
<tr>
<td><em>amp1</em>-9 <em>mpG92</em></td>
<td>26</td>
<td>165</td>
<td>15.8</td>
</tr>
</tbody>
</table>

### E. Floral organ numbers of *amp1 mp* double mutants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Range</th>
<th>Mean</th>
<th>Range</th>
<th>Mean</th>
<th>Range</th>
<th>Mean</th>
<th>Range</th>
<th>Mean</th>
<th>Range</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>amp1</em>-9</td>
<td>50</td>
<td>4</td>
<td>4.0</td>
<td>4</td>
<td>4.0</td>
<td>5-6</td>
<td>5.8±0.1</td>
<td>2</td>
<td>2.0</td>
<td>15-16</td>
<td>15.8±0.1</td>
</tr>
<tr>
<td><em>amp1</em>-9 <em>mpG92</em></td>
<td>40</td>
<td>0-4</td>
<td>2.4±0.1$^\dagger$</td>
<td>2-8</td>
<td>3.5±0.2$^\dagger$</td>
<td>1-4</td>
<td>2.7±0.2$^\dagger$</td>
<td>1-2</td>
<td>1.6±0.1$^\dagger$</td>
<td>7-15</td>
<td>10.2±0.2$^\dagger$</td>
</tr>
<tr>
<td><em>amp1</em>-8</td>
<td>50</td>
<td>4</td>
<td>4.0</td>
<td>4</td>
<td>4.0</td>
<td>5-6</td>
<td>5.9±0.1</td>
<td>2</td>
<td>2.0</td>
<td>15-16</td>
<td>15.9±0.1</td>
</tr>
<tr>
<td><em>amp1</em>-8 <em>mpBS62</em></td>
<td>50</td>
<td>2-4</td>
<td>3.7±0.1$^\dagger$</td>
<td>2-4</td>
<td>3.6±0.1$^\dagger$</td>
<td>4-6</td>
<td>5.0±0.1$^\dagger$</td>
<td>1-2</td>
<td>1.9±0.1</td>
<td>11-16</td>
<td>14.2±0.2$^\dagger$</td>
</tr>
</tbody>
</table>

* Values are range and mean number of floral organs produced±s.e.m.

### F. Apical pattern phenotypes of *amp1 mp* double mutants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Monocot %</th>
<th>Fused %</th>
<th>&gt; 2 cots %</th>
<th>Other %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>amp1</em>-11 <em>mpBS1354</em></td>
<td>88</td>
<td>23.9</td>
<td>14.8</td>
<td>61.3</td>
<td></td>
</tr>
<tr>
<td><em>amp1</em>-8 <em>mpBS62</em></td>
<td>181</td>
<td>2.7</td>
<td>3.9</td>
<td>92.8</td>
<td>0.6</td>
</tr>
<tr>
<td><em>amp1</em>-9 <em>mpG92</em></td>
<td>125</td>
<td>20.4</td>
<td>4.7</td>
<td>64.6</td>
<td>10.3</td>
</tr>
<tr>
<td><em>mpBS1354</em></td>
<td>92</td>
<td>50.0</td>
<td>4.3</td>
<td>44.6</td>
<td>1.1</td>
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<tr>
<td><em>mpBS62</em></td>
<td>74</td>
<td>35.1</td>
<td>8.1</td>
<td>56.8</td>
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<tr>
<td><em>mpG92</em></td>
<td>107</td>
<td>14.9</td>
<td>25.3</td>
<td>59.8</td>
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Genetic interaction between AMP1 and MP
Our observations suggest a model in which auxin-derived positional information through MP carves out meristematic niches by locally overcoming a general differentiation-promoting activity involving AMP1 (Fig. 3C). This model is consistent with the overall meristem-promoting activity of MP contrasted by the differentiation-promoting activity of AMP1, but clearly involves more than the superimposition of opposite controls. First, as explained above, the interaction cannot be reduced to the superimposition of opposite controls. Second, MP does not seem to overcome AMP1 activity by transcriptional downregulation of AMP1, because AMP1 transcript levels are unaffected in mp seedlings (Fig. 3D). Third, MP and AMP1 are unlikely to interact physically, as they are localized to different cellular compartments (see Fig. S4B,C in the supplementary material). This finding is also reflected in the absence of semidominant suppressive effects, which are frequently associated with direct interaction, in amp1 mp double mutants. Both gene products are, however, co-expressed in many locations and their respective pathways could therefore interact in those cells (see Fig. S5 in the supplementary material).

In summary, mutant phenotypes and expression patterns suggest that MP locally interferes with AMP1-promoted cell differentiation to maintain meristematic niches.

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Supplementary material
Supplementary material for this article is available at http://dev.biologists.org/cgi/content/full/134/14/2561/DC1

References

Fig. 3. Vascular systems in cotyledons of wild-type and mutant Arabidopsis seedlings. (A, B) Vascular phenotype categories: ‘straight’, referring to the formation of a single unbranched midvein; ‘branched’, a complete midvein plus some secondary venation; ‘circles’, complete midvein and at least one second-order vein has formed a complete loop. Frequencies of vascular phenotypic categories (shown in B) in various genotypes. Note the higher frequencies of more-complete vascular systems in amp1 mp double mutants as compared with mp mutants. One cotyledon per seedling was evaluated. (C) Scheme of AMP1 interaction with MP. As illustrated for cell fate acquisition in the leaf primordium, but applicable also to other locations, AMP1 functions as a universal negative regulator of meristem activity (promoter of cell differentiation). Locally interfering with AMP1 function, MP maintains cells in a procambial state by preventing the acquisition of mesophyll characteristics (green) along lines of elevated auxin levels and MP expression (yellow). (D) RT-PCR of AMP1 transcript abundance in wild-type and mpG92 seedlings at 7 days after germination (DAG). ACT7 was used as an internal control as described (Hardtke et al., 2004).


