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The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development

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The vascular tissues of flowering plants form networks of interconnected cells throughout the plant body. The molecular mechanisms directing the routes of vascular strands and ensuring tissue continuity within the vascular system are not known, but are likely to depend on general cues directing plant cell orientation along the apical–basal axis. Mutations in the *Arabidopsis* gene *MONOPTEROS* (*MP*) interfere with the formation of vascular strands at all stages and also with the initiation of the body axis in the early embryo. Here we report the isolation of the *MP* gene by positional cloning. The predicted protein product contains functional nuclear localization sequences and a DNA binding domain highly similar to a domain shown to bind to control elements of auxin inducible promoters. During embryogenesis, as well as organ development, *MP* is initially expressed in broad domains that become gradually confined towards the vascular tissues. These observations suggest that the *MP* gene has an early function in the establishment of vascular and body patterns in embryonic and post-embryonic development.

Keywords: auxin signalling/embryo pattern formation/organogenesis/plant cell axialization/transcriptional regulation

Introduction

Vascular tissues form ramified systems of continuous cell files, each made of elongated, interconnected cells (Steeves and Sussex, 1989; Lyndon, 1990). Vascular patterning is thought to depend on signals directing the routes of vascular strands as well as the oriented differentiation of each cell within the vascular system. The molecular nature of these signals is not known, but (canalized) flows of translocatable signal molecules, possibly involving the plant hormone auxin, have been implicated in vascular differentiation (reviewed in Shininger, 1979; Lyndon, 1990; Sachs, 1991; Nelson and Dengler, 1997).

Embryonic and post-embryonic development are tightly linked to the patterning of the internal provascular systems and are therefore thought to depend, at least in part, on common directional signals (Sachs, 1991; Cooke *et al.*, 1993). Although vascular tissues differentiate at predictable positions during normal development, adaptive responses to wounding or abnormal growth conditions demonstrate considerable flexibility of vascular patterning and enable investigation of the nature of the underlying signals. Local application of the plant hormone auxin has been shown to influence efficiently the vascular pattern in mature organs (Sachs, 1981). When applied at early stages, auxin, as well as chemical inhibitors of auxin transport, severely affects the architecture of the embryo (Liu *et al.*, 1993; Fischer and Neuhaus, 1996). These observations suggest an involvement of the plant hormone auxin in mechanisms co-ordinating the development of embryos and the respective vascular patterns.

A number of mutants affecting various aspects of the vascular pattern have been identified (reviewed in Freeling, 1992; Nelson and Dengler, 1997), but only two mutants have been described to affect vascular tissue continuity throughout the plant body. In the *Arabidopsis* mutant *lopped 1* (Carland and McHale, 1996), interruptions of vascular strands are associated with abnormal spiral growth of all organs and with gross abnormalities in leaf shape. Mutations in the gene *MONOPTEROS* (*MP*) interfere with the formation of the vascular system already in the embryo (Berleth and Jürgens, 1993). Early in embryogenesis, *mp* mutants lack centrally located provascular cells within a basal domain of the embryo. This domain gives rise to the hypocotyl (seedling stem) and the primary root (collectively referred to as the embryo axis; Figure 1A and C) (Berleth and Jürgens, 1993; Przemeck *et al.*, 1996). Both structures are completely missing in mutant embryos and seedlings (Figure 1B and D) and the mutant has therefore been classified as a potential embryo pattern mutant (Mayer *et al.*, 1991). Occasionally, *mp* mutant seedlings can produce adventitious roots enabling studies of mutant traits at post-embryonic stages. In all organs analyzed, cells within vascular strands appear incompletely differentiated and insufficiently interconnected (Przemeck *et al.*, 1996), and in all leaf organs the vascular system is reduced to higher order veins (for example, see Figure 1F and G). Furthermore, there are variable distortions in the formation of lateral organs, particularly in the inflorescence (Przemeck *et al.*, 1996). This abnormality is reminiscent of plants treated with chemical inhibitors of auxin transport (Okada *et al.*, 1991). Auxin transport is reduced in *mp* mutants, even when measured in stem segments of mutants from weak alleles that do not display marked vascular abnormalities (Przemeck *et al.*, 1996). Based on these observations, the *MP* gene has been proposed to mediate plant cell axialization in response to directional cues along the apical–basal axis (Przemeck *et al.*, 1996).

In this report we describe the isolation and molecular characterization of the *MP* gene. We show that the *MP* gene encodes a protein with features of a transcriptional regulator that is very likely to be capable of modulating gene activities in response to auxin signals. These

molecular properties, as well as the *MP* expression profile, are consistent with the mutant phenotype and suggest that *MP* influences embryo pattern formation as well as vascular development by mediating axialized behavior of plant cells in response to auxin cues.

Results

Isolation of the MP gene

The *MP* locus has been mapped to the upper part of the first chromosome (Berleth and Jürgens, 1993). Using restriction fragment length polymorphism markers (RFLP) from this region, we have localized the gene between markers *m59* and *g2395* (Hardtke and Berleth, 1996). Within this region, ~2200 kbp of contiguous genomic DNA from libraries of Yeast Artificial Chromosome (YAC) clones were isolated (Figure 2A). Mapping of RFLPs detected by DNA fragments from the chromosome walk enabled us to assign the *MP* gene to a single YAC that was utilized to identify and map new RFLP loci in the immediate vicinity of the *MP* locus. Based on these new RFLP loci, a local chromosome walk of cosmid and Bacterial Artificial Chromosome (BAC) clones was generated, encompassing a genetic interval defined by two recombination events on either side of the *MP* gene (Figure 2A). Eight classes of non-overlapping cDNA clones were identified within this interval.

To detect allele-specific RFLPs, cDNA clones representing the eight presumed transcription units in the region were hybridized to genomic Southern blots of mutant DNA. Two overlapping cDNA clones (*KL1* and *KS18*) identified an allele-specific DNA polymorphism (Figure 2B). The longer cDNA clone, *KL1* (2.7 kbp) was extended by RACE–PCR to obtain sequence information for 3.1 kbp of transcribed DNA. Both cDNAs detected a single low-abundance 3.2 kbp transcript on poly(A)$^+$ RNA blots (data not shown) suggesting that the cDNA sequence represents the full-length transcript. Comparison of the cDNA and corresponding genomic sequences indicated a transcription unit of 13 exons spread over a genomic interval of ~4.5 kbp (Figure 2C). We confirmed that this transcription unit represents the *MP* gene by analyzing the genomic sequences from six *mp* alleles. Direct sequencing of PCR products revealed that all six alleles had either stop or frame-shift mutations at different positions within the predicted coding sequence (Figure 2C). Based on the perfect cosegregation and the existence of deleterious point mutations in six mutant alleles, we conclude that the isolated transcription unit represents the *MP* gene.

Sequence analysis and nuclear localization of the presumptive MP product

The open reading frame of the *MP* gene encodes a predicted protein product of 902 amino acids and contains three stretches of similarity with the recently described *Arabidopsis* transcription factor ARF1 (Ulmasov *et al.*, 1997). Sequence similarities between MP and ARF1 are particularly pronounced within a presumptive DNA binding domain (76% similarity between residues 150–264, Figure 2E) that is related to Maize transactivator *Viviparous 1* (McCarty *et al.*, 1991) (Figure 2E). The MP sequence is most probably partially represented by cDNA *IAA24* (Ulmasov *et al.*, 1997) (865 out of 866 amino acids identical). The DNA binding domains of ARF1 and IAA24 have recently been shown to bind to the same functionally defined promoter elements of auxin-inducible genes (Ulmasov *et al.*, 1997), suggesting that these proteins regulate downstream genes in response to auxin signals. In the C-terminal region, the products of *MP, ARF1* and a larger group of otherwise unrelated auxin-inducible genes (Abel *et al.*, 1995), contain two additional stretches

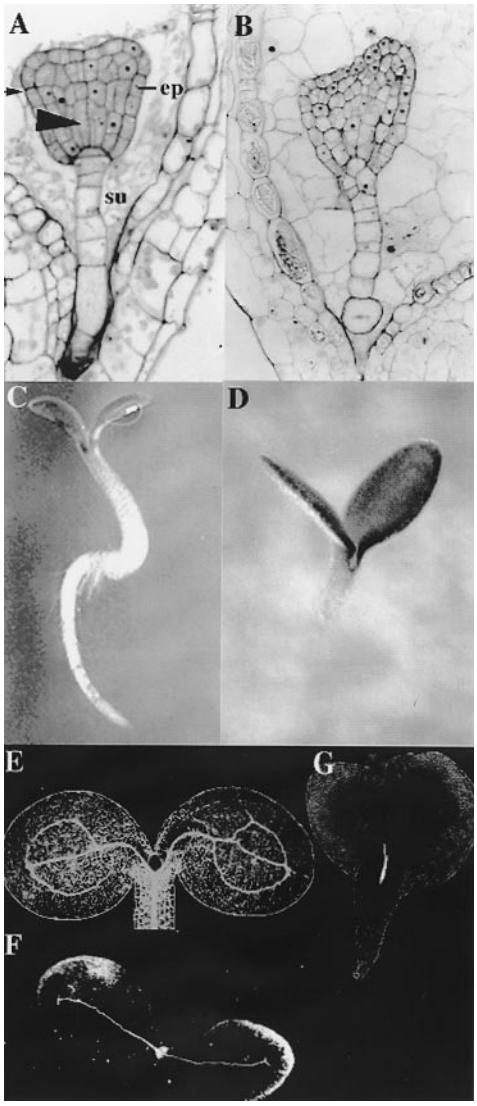
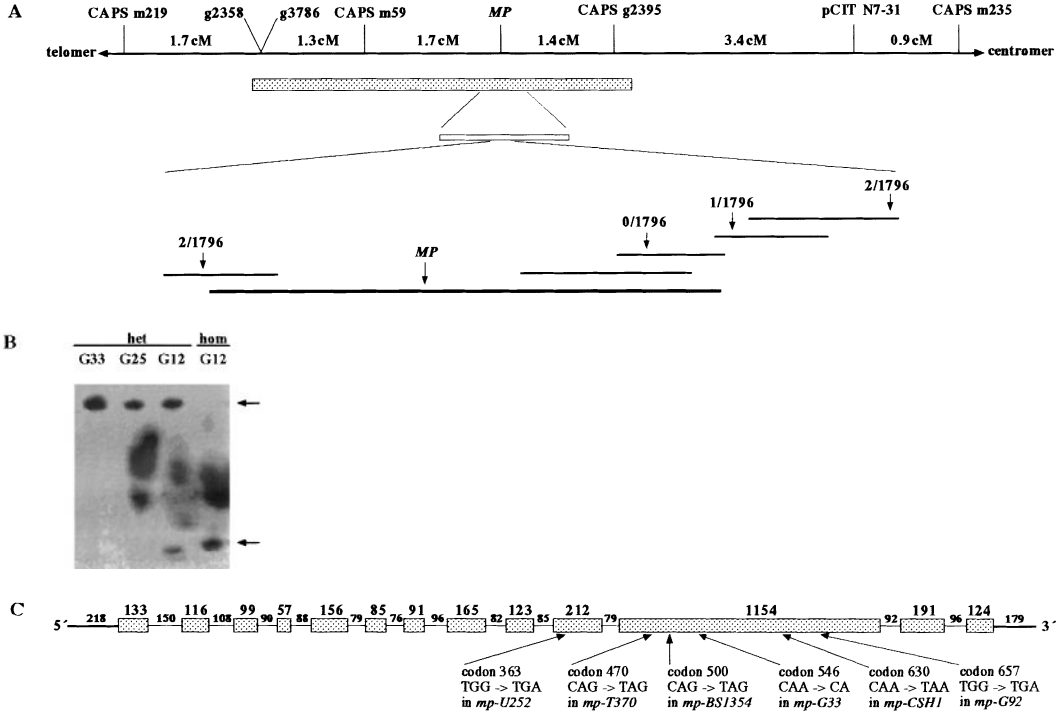


Fig. 1. (**A**) Wild-type embryos at the onset of heart-stage (cotyledons just emerged) display files of elongated cells in all subepidermal tissues of the incipient hypocotyl/root region (separated from apical region by an arrow in A). Arrowhead in (A) points at elongated cells of the incipient vascular system; ep, epidermis; su, suspensor. (**B**) Cells in the corresponding part of *mp* mutant embryos are neither elongated nor organized in files, while unrelated structures, such as the suspensor and the epidermis, are not affected. (**C**) The wild-type seedling consists of a shoot meristem (not visible), two cotyledons, the hypocotyl and the primary root. (**D**) In *mp* mutants cotyledons may be variably fused and the hypocotyl/root axis is replaced by a basal peg of unorganized tissue. (**E**) The vascular system in wild-type cotyledons comprises lobes extending from a central vascular strand. (**F**) Vascularization is reduced to central strands with small terminal branches in mutants of the weak allele *CSH1*. (**G**) Vascularization is further reduced to central veins or less in individuals of a strong allele, *U252*. Allele-specific differences are also reflected in a number of other defects, such as integrity of individual vascular strands and the frequency of cotyledon fusions (data not shown).



D

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MMASLSCVEDKMKTSCLVNGGGTITTTTSQSTLLEEMKLLKDQSGTRKPV 50
INSELWHACAGPLVCLPQVGSLVYYFSQGHSEQVAVSTRRSATTQVPNYP 100
NLPSQLMCQVHNVTLHADKDSDEIYAQMSLQPVHSERDVFPVPDFGMLRG 150
SKHPTEFFCKTLTASDTSTHGGFSVPRRAAEKLFPPLDYSAQPPTQELVV 200
RDLHENTWTFRHIYRGQPKRHLLTTGWSLFVGSKRLRAGDSVLFIRDEKS 250
QLMVGVRRANRQQTALPSSVLSADSMHIGVLAAAAHATANRTPFLIFYNP 300
RACPAEFVIPLAKYRKAICGSQLSVGMRFGMMFETEDSGKRRYMGTIVGI 350
SDLDPLRWPGSKWRNLQVEWDEPGCNDKPTRVSPWDIETPESLFIFPSLT 400
SGLKRQLHPSYFAGETEWGSLIKRPLIRVPDSANGIMPYASFPSMASEQL 450
MKMMMRPHNNQNVPSFNSEMQQNIVMGNGGLLGDMKMQQPLMMNQKSEMV 500
QPQNKLTVNPSASNTSGQEQNLSQSMSAPAKPENSTLSGCSSGRVQHGLE 550
QSMEQASQVTTSTVCNEEKVNQLLQKPGASSPVQADQCLDITHQIYQPQS 600
DPINGFSFLETDELTSQVSSFQSLAGSYKQPFILSSQDSSAVVLPDSTNS 650
PLFHDVWDTQLNGLKFDQFSPLMQQDLYASQNICMSNSTTSNILDPPLSN 700
TVLDDFCAIKDTDFQNHPSGCLVGNNNTSFAQDVQSQITSASFADSQAFS 750
RQDFPDNSGGTGTSSSNVDFDDCSLRQNSKGSSWQKIATPRVRTYIKVQK 800
TGSVGRSIDVTSFKDYEELKSAIECMFGLEGLLTHPQSSGWKLVYVDYES 850
DVLLVGDDPWEEFVGCVRCIRILSPTEVQQMSEEGMKLLNSAGINDLKTS 900
VS
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E

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VP1   ADKN-LRFLLQKVLKQSDVGSLGRIVLPKKEAEVHLPELKTR---DGISIPMEDIGTSRVWNMRYRFWPNNKSRMYLLENT-GEFVRSNELQEGDFIVIYSDVKSGKYLIRGVKVRPPPAQE
MP    GSKHPTEFFC-KTLTASDTSTHGGFSVPRRAAEKLFPPLDYSAQPPTQELVVRDLHENT-WTFRHIYR--GQPKRHLLTTGWSLFVGSKRLRAGDSVLFIRDEKSQLMV--GVR-RANRQQT
ARF1  PEKCTVHSFC-KTLTASDTSTHGGFSVLRRHADDCLPPLDMSQQPPWQELVATDLHNSE-WHFRHIFR--GQPRRHLLTTGWSVFVSSKKLVAGDAFIFLRGENKELRV--GVR-RHMRQQT
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Fig. 2. (**A**) Map of the *MP* genomic region with adjacent RFLP markers (top; Chang *et al.*, 1988; Nam *et al.*, 1989), approximate genetic distances and the extent of the chromosome walk (shaded rectangle; Hardtke and Berleth, 1996). The approximate positions of YAC clone CIC8C5 (Creusot *et al.*, 1995) (~450 kbp, open rectangle) within the chromosome walk and of the aligned cosmid (thin lines) and BAC (bold line) clones within CIC8C5 are indicated below. Numbers give genetic distances in cM (top) and the fraction of recombinations in chromosomes from a total of 1796 meiotic products (bottom). The aligned cosmid and BAC clones span a region of roughly 60 kbp. (**B**) Southern blot analysis. DNA from plants heterozygous (het) or homozygous (hom) for the indicated *mp* mutant alleles hybridized with ^{32}P-labeled *MP* cDNA clone *KS 18* (1.8 kbp). The three alleles, *G33*, *G25* and *G12*, were induced in *Col-0* by gamma-ray mutagenesis. The single band in *G33* and *G25* represents the absence of detectable restriction site alterations (for *G33* consistent with the single base pair deletion shown in Figure 2C). Arrowheads mark the positions of the bands affected by allele specific restriction site polymorphism in allele *G12*. The molecular lesion in the gamma-ray-induced allele *G12* was not further characterized, as it appears to represent a larger chromosomal defect associated with multiple RFLPs (data not shown). (**C**) Structure of the *MP* transcription unit. The diagram depicts introns (thin lines) and exons (5′ and 3′ untranslated regions, bold lines; ORF, shaded boxes). Sizes are given in nucleotides. The only long open reading frame is preceded by six in-frame stop codons. Arrows below mark the positions of sequence alterations in six *mp* alleles. Codon positions refer to the predicted protein product shown in (D). (**D**) Predicted amino acid sequence (single-letter code) of the MP protein product (*Col* wild-type). Numbers to the right indicate the position relative to the putative translational start site. Positions of the potential functional domains are marked by rectangles on top: DNA-binding domain (shaded), nuclear localization sequences (filled, domains of bipartite NLSs connected by thin line), a potential helix–loop–helix domain predicted by structural features (Rost and Sander, 1994) (hatched) and two stretches of similarity to *ARF1* (Ulmasov *et al.*, 1997) and to a number of auxin inducible genes (Abel *et al.*, 1995) (open). (**E**) Amino acid sequence comparison (single-letter code). An alignment is shown for the deduced amino acid sequences from the gene products of VP1 (McCarty *et al.*, 1991), MP (shaded rectangle in D) and ARF1 (Ulmasov *et al.*, 1997). Identical and functionally conserved amino acids are indicated by # and *, respectively.

of similarity. These two regions (residues 794–827 and 838–880, Figure 2D) (Ulmasov *et al.*, 1997) have (for ARF1) been implicated in protein–protein interaction.

Interestingly, all six *mp* mutations characterized appear not to affect the DNA-binding domain (Figure 2C). Since all available *mp* mutants have been identified at the seedling stage, this raises the possibility that true null-alleles are missing due to early embryonic lethality. All *mp* alleles analyzed delete the C-terminal stretches of homology and could thus reflect the requirement of protein interactions for full gene activity. Furthermore, premature stop codons at different positions in the central portion of the predicted MP protein are associated with two distinguishable phenotypes. Mutants of alleles *CSH1* and *G92* (premature stop codons at residues 630 and 657, respectively; Figure 2C) display intermediate vascular defects (Figure 1F), while stronger phenotypes are observed in alleles with stop codons (or frame-shift mutations) at earlier positions (Figures 1G and 2C). Distinguishable weak and strong phenotypes have also been reported for previously isolated *mp* alleles (Berleth and Jürgens, 1993).

The predicted MP product contains three potential nuclear localization signals (NLSs) within the presumed DNA-binding domain (Figure 2D). One of these NLSs is of bipartite structure, while two show similarity with the MATα-class of NLS (Raikhel, 1992). Two further potential NLSs were identified outside the DNA-binding domain (Figure 2D). To test the ability of the *MP* product to exert a nuclear function and to delimit sequences essential for nuclear import, we have assessed the capacity of MP protein domains to confer nuclear localization to an attached β-glucuronidase (GUS) reporter gene product by assaying transient expression in onion epidermis cells. A blue precipitate monitoring GUS activity was confined to the nuclei in cells transformed with an MP–GUS fusion construct, containing residues 4–289 of the predicted ORF, indicating the functionality of at least one of three proposed NLSs within the DNA-binding domain (Figures 2D and 3A). By contrast, a second MP–GUS fusion protein containing residues 297–901 of the predicted MP product was not selectively imported into the nucleus (Figures 2D and 3B). We conclude that functional NLSs are located in the N-terminal part of the protein and that nuclear import is not dependent on possible molecular interactions of the C-terminal domains that are missing in mutant gene products.

Expression of the MP gene in embryonic and post-embryonic development

In RNA blot analyses, low levels of *MP* transcripts were detected in all major organs (data not shown) consistent with ubiquitous vascular distortions (Przemeck *et al.*, 1996). In order to better correlate the expression pattern to the phenotypic defects, we determined *MP* transcript distribution by *in situ* hybridization to tissue sections of embryos and plant organs. In early globular embryos, *MP* transcripts were present in all subepidermal cells (Figure 4A), while in heart-stage embryos expression was confined to broad, yet more central domains along the midlines of the cotyledons, as well as of the embryo axis (Figure 4B). Expression was further restricted to the centers of embryonic organs in early torpedo-stage embryos (Figure 4C) and was ultimately confined to provascular tissues of the differentiating vascular strands in nearly mature embryos (Figure 4D). Similarly, *MP* was expressed in broad domains in emerging determinant shoot organs (Figure 4F and H), while in more mature organs expression was restricted to procambial and possibly to some differentiated vascular regions (Figure 4F, G, L and M). In early leaf primordia, ubiquitous (yet subepidermal) expression was observed that became gradually restricted to vascular tissues upon leaf maturation (Figure 4G). In early flower primordia, *MP* was expressed in all whorls (Figure 4H), but was confined to vascular tissues in maturing flower organs. At late stages of flower development, expression was most pronounced in the gynoecium, particularly in developing ovules including the funiculi (Figure 4K). In mature roots, *MP* expression was detectable only within the central cylinder (Figure 4I).

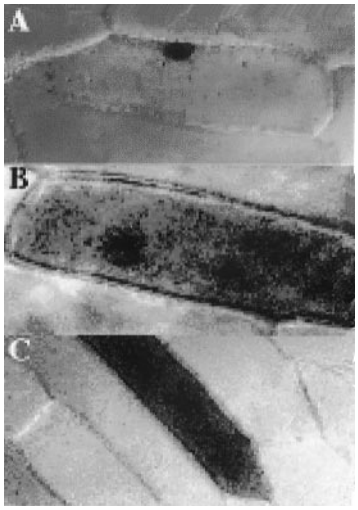

Fig. 3. Expression of *MP*–GUS fusion proteins in onion epidermis cells. (**A**) Expression of a fusion protein containing the presumed DNA-binding domain with three potential NLSs (residues 4–289) of the predicted *MP* protein fused to the N-terminus of the GUS gene. (**B**) Expression of a similar construct as in (A), comprised of most of the remaining coding sequence (residues 297–901) of the predicted *MP* product including two more NLS-related sequences that may also constitute another bipartite NLS. (**C**) Control of expression of the GUS gene in the same vector.

Discussion

In this study, we have determined and analyzed the molecular identity and expression profile of the *MP* gene product. The *MP* gene had initially been identified by mutations that severely distort the embryonic pattern (Berleth and Jürgens, 1993) and subsequent studies suggested that the embryonic defects reflect a more general incapacity of mutant cells to respond to apical–basal axial cues that are instrumental for both embryo axis formation and vascular development (Przemeck *et al.*, 1996). Here we show that the *MP* gene encodes a protein partially represented by a recently identified transcriptional regulator (Ulmasov *et al.*, 1997) whose binding properties match the expectations of widely accepted concepts of plant apical–basal signaling (Sachs, 1991). We further show that *MP* expression in early embryo and organ development coinincides with axial cell orientation and later converges towards the routes of vascular differenti-

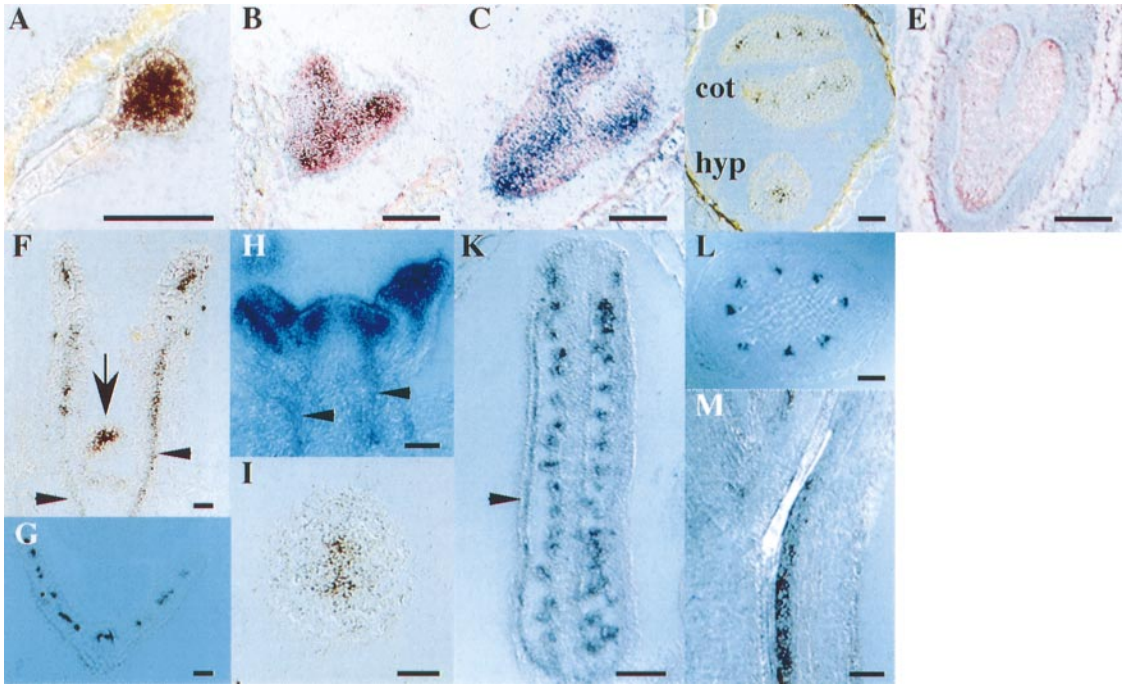

Fig. 4. Expression pattern of *MP* mRNA in wild-type (*Col-0*) embryos and plants. *In situ* hybridization with *MP* sense (**E**) and antisense (A–D, F–M) probe. (A–D) Progressive central confinement of *MP* expression in embryos from early-globular (**A**) through early-heart (**B**) and early-torpedo (**C**) stages (median longitudinal sections in A–C). In the nearly mature embryo [cross-section in (**D**)] *MP* expression is restricted to provascular tissues in the cotyledons and in the hypocotyl. (**F**) Median longitudinal section of shoot apical meristem of a 7-day-old seedling showing broad subepidermal *MP* expression domains in young leaf primordia (arrow) and predominantly procambial expression in more mature leaves (arrowheads). (**H**) Longitudinal section of an inflorescence apex. Broad, strong *MP* expression in flower and floral organ primordia of different stages and weaker procambial expression (arrowheads). No detectable expression in the inflorescence meristem apex. (**K**) Gynoecium displaying *MP* expression in immature ovules and vascular tissues (arrowhead). (G–M) *MP* expression confined to the central root cylinder (**I**) and to vascular bundles in a mature cauline leaf (**G**) and inflorescence stem [cross-section in (**L**), longitudinal section in (**M**)]. Abbreviations: cot, cotyledons; hyp, hypocotyl. Scale bars: 50 μM in A-E, H, K-M; 200 μM in F, G, I.

ation, consistent with an early function in vascular strand formation.

The role of the MP gene in vascular development

The molecular mechanisms underlying vascular patterning and differentiation are poorly understood. Genetic approaches in *Arabidopsis* have been hampered by the difficulty of visualizing the vascular system in mutant screens and only a very small number of mutants have been isolated, either based on anatomical defects (Turner and Somerville, 1997) or by associated morphological abnormalities (Berleth and Jürgens, 1993; Carland and McHale, 1996). The *Arabidopsis* mutant *mp* was initially recognized by its conspicuous seedling phenotype lacking all structures derived from the basal domain of the embryo (Mayer *et al.*, 1991). This localized defect suggested a region-specific organizing function of the *MP* gene during plant embryo pattern formation (Mayer *et al.*, 1991; Berleth and Jürgens, 1993). When analyzed at the anatomical level, however, *mp* mutant organs of all developmental stages display a unique type of vascular defect characterized by an overall reduction of vascular tissues and by incomplete tissue continuity within vascular strands (Przemeck *et al.*, 1996). No general cellular defects were detected, and even within the vascular system all classes of differentiated cells can be observed (Przemeck *et al.*, 1996). The *mp* gene function thus seems to be required to mediate the integrated formation of vascular cell files rather than to promote particular events during vascular differentiation. In this study we show that *MP* is very specifically expressed in the vascular tissues at all stages of vascular maturation. Unlike other genes implicated in vascular development, however, this tissue-specific signal is preceded by an extremely early expression in far broader domains that become gradually confined towards the sites of vascular differentiation. These observations suggest an early function of the *MP* gene in organ initiation and the gradual sharpening of the *MP* expression domains seem to reflect genetic interactions establishing the vascular pattern during organogenesis.

Axial cues in vascular and organ development

The distribution of *MP* transcripts in developing embryos does not support the earlier concept of a complex region-specific organizing role of the gene in the basal domain of the embryo. Rather, the molecular data support the view that the basal focus of the mutant embryonic phenotype results from a localized requirement for axial information at the onset of hypocotyl/root axis formation in the early embryo. Vascular differentiation and the generation of the hypocotyl/root body region appear to be developmentally linked and may thus be related at the cellular level. In fact, the general concept of an apical–basal signal flux underlying oriented cell behavior has not been restricted to vascular strand formation, but has also been applied to cell orientation in primordia (Sachs, 1991; Cooke *et al.*, 1993). Assuming that the initiation of axiality is more sensitive than its maintenance, it seems plausible

that the generation of continuous cell files from previously isodiametric cells, as it occurs in the early embryo or during lower-order vein initiation in leaf blades, is particularly dependent on proper perception of axial signals. These structures are most severely affected in *mp* mutants.

Irrespective of the molecular nature of these signals, the *mp* phenotype in alleles of different strength demonstrates that vascular and embryonic pattern formation are correlated. Mutants carrying weak *mp* alleles display a spectrum of seedling phenotypes, including those with short stretches of basal vascular tissue (Berleth and Jürgens, 1993). Analysis of these mutants revealed a tight correlation between the formation of short stretches of vascular tissue and the development of corresponding hypocotyl stumps (T.Berleth, unpublished data). Thus, vascular strand formation and basal organogenesis in the embryo might be directed by common underlying apical–basal cues.

The precise role of auxin in apical–basal signaling, suggested by a number of classical experiments, remains to be established. This developmental role may have not been fully addressed by genetic analyses of auxin functions, since auxin perception mutants have mainly been identified based on reduced auxin responses of adult plants (for review see Estelle and Klee, 1994; Hobbie *et al.*, 1994). Experiments involving the local application of indole-3-acetic acid (IAA, the major form of auxin in higher plants) and chemical inhibition of auxin transport have implicated an apical–basal flux of auxin in the formation of vascular strands (reviewed in Sachs, 1981). Furthermore, seedling defects similar to those of *mp* mutants have recently been described for the *Arabidopsis* mutant *auxin resistant 6* (Hobbie, 1997) and impaired embryonic symmetry, similar to cotyledon fusions observed in *mp* embryos, have been described for *Brassica juncacea* embryos treated with chemical inhibitors of auxin transport (Liu *et al.*, 1993). Moreover, cotyledon fusions as well as [*mp* related (Przemeck *et al.*, 1996)] spike-like inflorescences have been reported for the *Arabidopsis* mutant *pin formed* (Okada *et al.*, 1991). The *pin formed* mutant is impaired in the polar transport of auxin, as is the *mp* mutant (Przemeck *et al.*, 1996). These correlations, as well as the recently demonstrated capacity of the *MP* (*IAA24*) gene product to bind to functional auxin responsive promoter elements (Ulmasov *et al.*, 1997), suggest that developmental auxin signals could be relayed by the *MP* gene product. The possibility can be tested once the authentic target genes of the *MP* product have been identified. Notably, a number of structurally related presumptive transcription factors have recently been identified (Ulmasov *et al.*, 1997). The *MP* gene constitutes a member of this class of genes with genetically defined functions, and its further characterization should facilitate the analysis of the molecular signaling context (including the role of auxin), as well as genetic dissection of embryo and vascular development.

Materials and methods

High-resolution mapping of the MP region

Cosmid clones homologous to YAC CIC8C5 (Creusot *et al.*, 1995) were obtained by screening a genomic library from *Arabidopsis* ecotype *Landsberg erecta* (*Ler*, Meyer *et al.*, 1994) with DNA of this YAC eluted from pulsed-field gels. Nine cosmid clones were used to search for RFLPs between *Arabidopsis* ecotypes *Landsberg erecta* and *Niederzenz*. Six RFLPs were detected and mapped relative to meiotic recombination breakpoints in the region (data not shown). A local chromosome walk was initiated bidirectionally from a cosegregating cosmid (0/1796 recombinant meiotic products) aligning cosmid and BAC clones [from *Eco*RI partially digested *Columbia-0* (*Col-0*) genomic DNA; a not-ordered small-insert library was kindly provided by T.Altmann, Golm].

Isolation of cDNAs

Positive *MP* cDNA clones were present at a frequency of 4×10^{-6} in a library from etiolated seedlings (Kieber *et al.*, 1993). A likely full-length *MP* cDNA sequence was obtained by RACE PCR extension (5′3′-RACE-kit, Boehringer) of the longest available cDNA clone (*KL1*, 2.7 kbp). The length of the cDNA sequence (3.1 kbp) is consistent with the transcript size detected on poly(A)$^+$ RNA blots (~3.2 kbp).

Sequencing of mutant alleles

The extended cDNA and genomic DNA sequences of *Ler*, as well as *Col-0* wild-type strains and of mutant alleles *U252*, *CSH1*, *T370*, *G92* (*Ler* background), *BS1354* and *G33* (*Col-0* background), were determined by direct sequencing of PCR products generated with *Pfu* DNA polymerase (Stratagene, LaJolla) from two independent DNA preparations. The phenotype and origin of some of the mutant lines have been described in Berleth and Jürgens (1993) and Przemeck *et al.* (1996).

Transient transformation of onion epidermis cells

Constructs for transient expression were generated by inserting *MP* cDNA fragments in-frame between a translational start ATG and the GUS reporter gene moiety in vector *pNT160* (Boehm *et al.*, 1995). Transformation of onion epidermis cells and histochemical staining was performed as described (Varagona *et al.*, 1992) using a PDS1000 helium particle gun (Bio-Rad, Hercules).

In situ localization of transcripts in tissue sections

To synthesize the *MP* antisense probe, three cDNA fragments corresponding to nucleotide residues 210–825, 1281–1979 and 2659–3102 (maximum similarity to any data base sequence: 38% within a small interval) were subcloned in *pSP72* (Promega), linearized by digestion with *Eco*RI and transcribed with SP6 polymerase in the presence of [^{35}S]-UTP. Preparations of tissue sections, hybridization and exposure were performed as described (Drews *et al.*, 1991). The sections were exposed with emulsion for ~3 weeks.

Accession numbers

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