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The auxin-insensitive *bodenlos* mutation affects primary root formation and apical-basal patterning in the *Arabidopsis* embryo

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SUMMARY

In *Arabidopsis* embryogenesis, the primary root meristem originates from descendants of both the apical and the basal daughter cell of the zygote. We have isolated a mutant of a new gene named *BODENLOS* (*BDL*) in which the primary root meristem is not formed whereas post-embryonic roots develop and *bdl* seedlings give rise to fertile adult plants. Some *bdl* seedlings lacked not only the root but also the hypocotyl, thus resembling *monopteros* (*mp*) seedlings. In addition, *bdl* seedlings were insensitive to the auxin analogue 2,4-D, as determined by comparison with *auxin resistant1* (*axr1*) seedlings. *bdl* embryos deviated from normal development as early as the two-cell stage at which the apical daughter cell of the zygote had divided horizontally instead of vertically. Subsequently, the uppermost derivative of the basal daughter cell, which is normally destined to become the hypophysis, divided abnormally and failed to generate the quiescent centre of the root meristem and the central root cap. We also analysed double mutants. *bdl mp* embryos closely resembled the two single mutants, *bdl* and *mp*, at early stages, while *bdl mp* seedlings essentially consisted of hypocotyl but did form primary leaves. *bdl axr1* embryos approached the *mp* phenotype at later stages, and *bdl axr1* seedlings resembled *mp* seedlings. Our results suggest that *BDL* is involved in auxin-mediated processes of apical-basal patterning in the *Arabidopsis* embryo.

Key words: *Arabidopsis thaliana*, *BODENLOS (BDL)*, Embryogenesis, Apical-basal patterning, Auxin

INTRODUCTION

The basic body organisation of higher plants is established during embryogenesis (for review, see Laux and Jürgens, 1997). The apical-basal axis of the seedling consists of a sequence of distinct structures: shoot meristem, cotyledons, hypocotyl, root and root meristem. In *Arabidopsis*, the origins of these structures have been traced back to specific cells or groups of cells in the early embryo, both histologically and by clonal analysis (Mansfield and Briarty, 1991; Jürgens and Mayer, 1994; Scheres et al., 1994). The current view is that the apical-basal pattern is established in steps which involve both asymmetric cell division and cell-cell interaction (Mayer and Jürgens, 1998). Initially, the zygote divides asymmetrically to give an embryogenic apical and an extra-embryogenic basal daughter cell. The apical daughter cell produces, by three rounds of stereotyped divisions, two tiers each of four cells which found the apical and central regions of the embryo, respectively. The basal daughter cell produces, by a series of horizontal divisions, a file of 6-9 cells of which all but the uppermost one form the extra-embryonic suspensor. The uppermost cell of the file becomes the hypophysis, or founder cell of the basal region of the embryo. The three regions of the embryo display different cell division patterns during the globular stages of embryogenesis. The apical and the central region give rise to the shoot meristem and the hypocotyl, respectively. However, adjacent regions, i.e. apical and central or central and basal, are thought to interact in the formation of the cotyledons and the root meristem, respectively.

The current view of apical-basal pattern formation is largely based on the analysis of mutant embryo phenotypes. *gnom* mutations affect the division of the zygote and destabilise the entire axis (Mayer et al., 1993; Vroemen et al., 1996) whereas other mutations have more restricted effects. *gurke* (*gk*) embryos become visibly abnormal at the heart stage when they fail to form cotyledon primordia (Torres Ruiz et al., 1996). *fackel* (*fk*) embryos appear to be defective in hypocotyl development such that the root seems directly attached to the cotyledons (Mayer et al., 1991). *hobbit* (*hbt*) embryos become recognisably abnormal at the 2- or 4-cell stage when the uppermost derivative of the basal daughter cell of the zygote divides vertically rather than horizontally (Willemsen et al., 1998). The hypophysis is not established and no functional root meristem forms in the embryo or during lateral root development. *monopteros* (*mp*) embryos are abnormal from the octant stage and later on fail to form hypocotyl, root and root meristem (Berleth and Jürgens, 1993). Analysis of *mp* post-embryonic development indicated that polar transport of auxin along the stem was reduced (Przemeck et al., 1996). The deduced MP protein (Hardtke and Berleth, 1998) is related to a transcription factor, ARF, that binds to auxin-response elements (Ulmasov et al., 1997). These findings raised the

possibility that auxin may be involved in apical-basal patterning.

Independent evidence for a role of auxin in embryogenesis was provided by experimental studies on explanted embryos. In the indian mustard *Brassica juncea*, auxin transport inhibitors and agonists applied to globular or heart-stage embryos had strong though variable effects on the apical-basal pattern of the seedling, resembling *gnom* or *mp* phenotypes (Hadfi et al., 1998). Similar effects were noted in wheat embryos (Fischer et al., 1996, 1997). Furthermore, inhibition of auxin polar transport interfered with the establishment of bilateral symmetry in *Brassica juncea* embryos, thus mimicking an incompletely penetrant *Arabidopsis pin1* phenotype (Liu et al., 1993). *pin1* plants have been shown to display reduced polar transport of auxin (Okada et al., 1991). Similarly, *pinoid* (*pid*) mutants of *Arabidopsis* have fused cotyledons and show age-dependent defects in polar transport of auxin (Bennett et al., 1995).

We have isolated a mutant of a new gene, *BODENLOS* (*BDL*), that affects the development of the primary root meristem in *Arabidopsis* embryogenesis. The earliest deviation from normal was observed in the apical daughter cell of the zygote. Later on, the uppermost derivative of the basal daughter cell, the presumptive hypophysis, developed abnormally and did not give rise to the quiescent centre of the root meristem. Furthermore, *bdl* seedlings were auxin-insensitive, and *bdl* appeared to genetically interact with both *mp* and the auxin-resistant mutant, *axr1*. These data are consistent with a role for *BDL* in auxin signaling during apical-basal patterning of the *Arabidopsis* embryo.

MATERIALS AND METHODS

Plant growth conditions, plant strains and genetic crosses

Plants were grown as previously described (Mayer et al., 1993). The wild-type strain used as a control was the Landsberg *erecta* (L*er*) ecotype. The *bodenlos* (*bdl*) mutant allele was isolated on the basis of its seedling phenotype after EMS mutagenesis of L*er* seeds (U. Mayer, unpublished result). The transgenic GUS-expressing line M276/7 kindly provided by V. Hecht and P. Gallois was used as a root cap-specific marker (Devic et al., 1995). Seeds homozygous for the *axr1-12* allele (Lincoln et al., 1990) were obtained from the *Arabidopsis* Biological Resource Center Ohio, USA. *fk*-X224 (Mayer et al., 1991) and *mp*-U55 (Berleth and Jürgens, 1993) have been described previously. Genetic crosses for constructing double mutants, for introducing the root-cap marker into the *bdl* line and for mapping the *bdl* mutation were done as previously described (Mayer et al., 1993).

Recombination mapping

The *bdl* allele was mapped against a set of molecular markers (CAPS, SSLP). *bdl*/BDL plants (L*er* ecotype) were crossed with Columbia (Col) wild-type plants, and the F_1 *bdl* (L*er*)/*BDL* (*Col*) plants were selfed to give an F_2 mapping population. F_2 seeds were surface-sterilised and plated on agar plates. Seven days after germination, *bdl* seedlings were selected and transferred to agar plates with MS medium (Duchefa, Haarlem, Netherlands). DNA was extracted from two leaves of individual *bdl* plantlets as described previously (Lukowitz et al., 1996) and amplified by PCR with primers for CAPS markers *PHYB* (Mayer et al., 1998), m235 (Hardtke and Berleth, 1998), *GL1* and PG11, and for SSLP markers nga59, nga63, nga111, nga168, nga6, nga8, nga225 and nga76 (Bell and Ecker, 1994; primers from Research Genetics Inc., Huntsville, USA). The markers have been localised on the RI map of Lister and Dean (1993; http://nasc.nott.ac.uk/new_ri_map.html). The frequency of recombination between *bdl* and each marker was determined as the number of recombinant chromosomes divided by twice the total number of chromosomes in F_2 *bdl* plants. With the exception of nga59 (4%), nga63 (4%) and m235 (20%), all markers gave recombinant frequencies close to 50%, indicating independent segregation.

Phenotypic analysis

For the analysis of seedling phenotypes, seeds were plated on 0.3% agar containing 50 μg/ml ampicillin, stored for 3 days at 4°C in the dark and then germinated in the light (constant illumination, 25°C). Seedling phenotypes were analysed about 7 days after germination, using an Olympus stereoscope equipped with an Olympus M4-Ti camera.

For histological analysis of embryos, ovules were processed as described before (Mayer et al., 1993). Whole-mount preparations were used for quantitative analysis of embryo and seedling phenotypes. Embryos or 7-day old seedlings were mounted in a mixture of chloralhydrate/distilled water/glycerol (8:3:0.5), incubated for 45 minutes at 4°C, cleared at room temperature for at least 60 minutes and viewed with Nomarski or dark-field optics using a Zeiss Axiophot microscope. Photographs were taken with a Kodak Ektachrom 64T film and processed with Adobe Photoshop 3.05 and Aldus Freehand 7.01 to create figures.

For GUS staining, bent-cotyledon stage embryos were incubated in staining buffer for 3 days at 37°C in the dark: 100 mM sodium phosphate pH 7.0, 10 mM EDTA, 3 mM ferricyanide, 0.1 % (v/v) Triton X-100, 2 mM X-Gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronide; Duchefa) dissolved in N-dimethyl-formamide (Sundaresan et al., 1995). Starch granules in the central root cap of 4-day old seedlings were visualised by lugol staining (Willemsen et al., 1998).

Tissue culture

Seeds from mutant lines were surface-sterilised in 70% ethanol for 1 minute and then in 5% calcium hypochlorite/0.15% Tween 20 for at least 30 minutes, rinsed with sterile water until all calcium hypochlorite was removed and plated on growth medium (GM) containing 0.5% agar, 1% sucrose, half-strength MS salts (Murashige and Skoog, 1962), 50 μg/ml ampicillin, 0.5 g/l MES pH 5.8. After germination, the seedlings were kept on this medium for 7 days (constant illumination, 25°C), then transferred to new GM plates and, after root formation, to soil.

Test for auxin insensitivity

To determine the response of *bdl*, *axr-1* and L*er* seedlings to 2,4-dichloro-phenoxyacetic acid (2,4-D), surface-sterilized seeds were germinated on GM supplemented with 0.2 mg/l 2,4-D. Seedling phenotypes were examined 7 days after germination. The ability of seedlings to form callus was assayed by germinating surface-sterilized *bdl*, *axr-1* and L*er* seeds on GM containing 1 mg/l 2,4-D and 0.25 mg/l kinetin, which induces callus formation in wild-type seedlings (T. Steinmann, personal communication). The response was evaluated 17 days after germination.

RESULTS

Genetics

The *bodenlos* (*bdl*) mutant was isolated on the basis of its seedling phenotype, from an EMS mutagenised L*er* population. Upon outcrossing to L*er* or *Col* wild-type, all F_1 seedlings were phenotypically normal, and half the F_1 plants produced 18% and 20% mutant seedlings upon selfing, respectively (Table 1). Although these values were smaller than

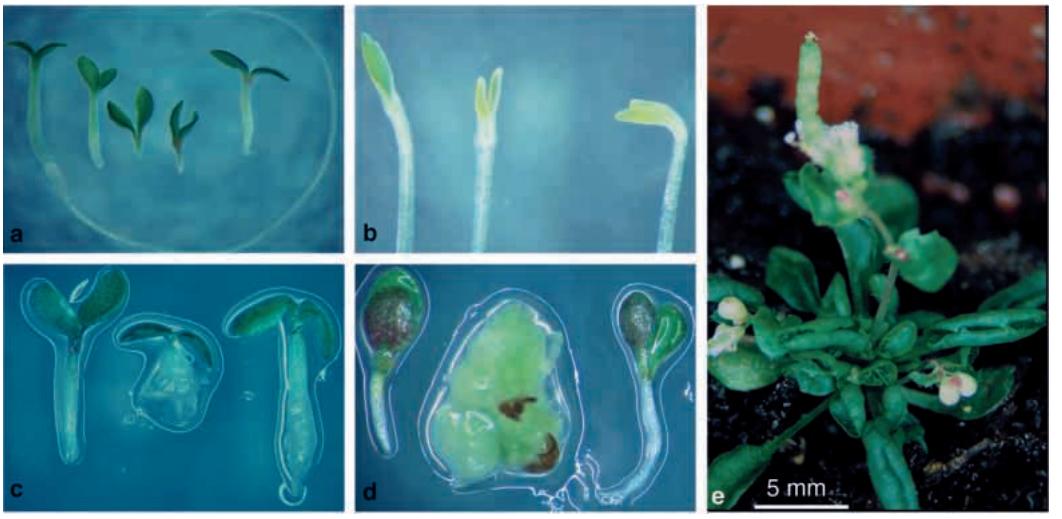

Fig. 1. *bdl* seedling and adult phenotypes. (a) *bdl* seedlings with strong phenotype (centre) flanked by those with a weak phenotype; wild-type control (left). (b) Apical end of dark-grown seedlings: *axr1*, *bdl*, wild-type (from left to right), only wild type displays an apical hook. (c) Effect of 2,4-D on root growth and hypocotyl elongation of seedlings: *bdl*, wild-type, *axr1* (from left to right); note that *axr1*, but not *bdl*, shows slight swelling of the hypocotyl. (d) Seedlings grown on callus-inducing medium: *bdl*, wild-type, *axr1* (from left to right); only wild-type forms callus. (e) *bdl* adult phenotype: stunted bushy plant with rolled-up leaves, normal-looking flowers and a maturing silique.

Table 1. Frequency of *bdl* seedlings from *bdl/BDL* plants of different ecotypes

Ecotype	Total no. of seedlings	Weak phenotype*	Strong phenotype*	One cotyledon	% mutant seedlings
L*er* (self)‡	1,203	138	65	9	18
L*er* (out)§	1,022	101	77	5	18
Col	522	81	21	0	20

*See Fig. 1a.
‡Line maintained by selfing.
§After outcross with L*er* wild type.

the expected 25% for a recessive mutation, we did not observe lethal embryos nor did seeds fail to germinate. *bdl* was mapped against molecular markers in a mapping population of 54 F_2 *bdl* seedlings. The results placed *bdl* on chromosome 1 in the interval defined by *nga59* and *nga 63* (for details, see Materials and Methods). Thus, the *bdl* phenotype appeared to result from a single recessive mutation.

bdl seedling phenotype

The *bdl* phenotype was somewhat variable. For practical purposes, we distinguished two classes designated 'weak' and 'strong' (Fig. 1a), with the latter representing a minority of 21 to 42% of mutant seedlings, depending on the genetic background (Table 1). Mutant seedlings with the strong phenotype lacked hypocotyl, root and primary root meristem, and the vasculature of the cotyledons was reduced (see Fig. 6d). Thus, the *bdl* strong phenotype was similar to *mp* (Berleth and Jürgens, 1993). Mutant seedlings with the weak phenotype lacked most of the primary root and the root meristem whereas the other parts of the seedling were fairly normal. Specifically, these *bdl* seedlings displayed root hairs at the basal end of the axis as are normally observed at the hypocotyl/root junction ('collet'; Scheres et al., 1994; see Fig. 6m). The *bdl* weak seedling phenotype thus resembled *hbt* (Willemsen et al., 1998). Although *bdl* seedlings did not show a primary root they did form secondary roots (see below).

Post-embryonic development

bdl seedlings continued to develop and gave rise to fertile adult plants (Fig. 1e). The *bdl* homozygous mutant plants were short with rolled-up leaves and had a bushy appearance, suggesting that apical dominance was reduced. In addition, the vasculature was defective (see Fig. 6l) and resembled that of *mp* adult plants that can be obtained by experimentally inducing adventitious roots in seedlings (Przemeck et al., 1996). However, *bdl* plants had normal-looking flowers and were self-fertile, producing 100% mutant seedlings. Post-embryonic development of *bdl* plants was sustained, in the absence of a primary root, by roots that formed in *bdl* seedlings (Fig. 2).

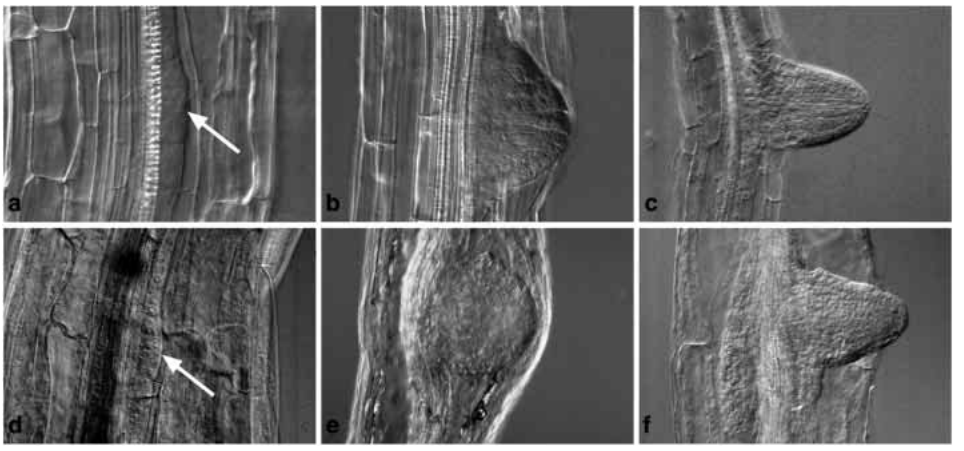

Fig. 2. *bdl* postembryonic root formation. (a-c) Wild-type lateral root; (d-f) *bdl* postembryonic root. (a,d) Primordium initiation; note bulging of cells undergoing periclinal division (white arrows). (b,e) Primordium organisation before emergence. (c,f) Growing root tip.

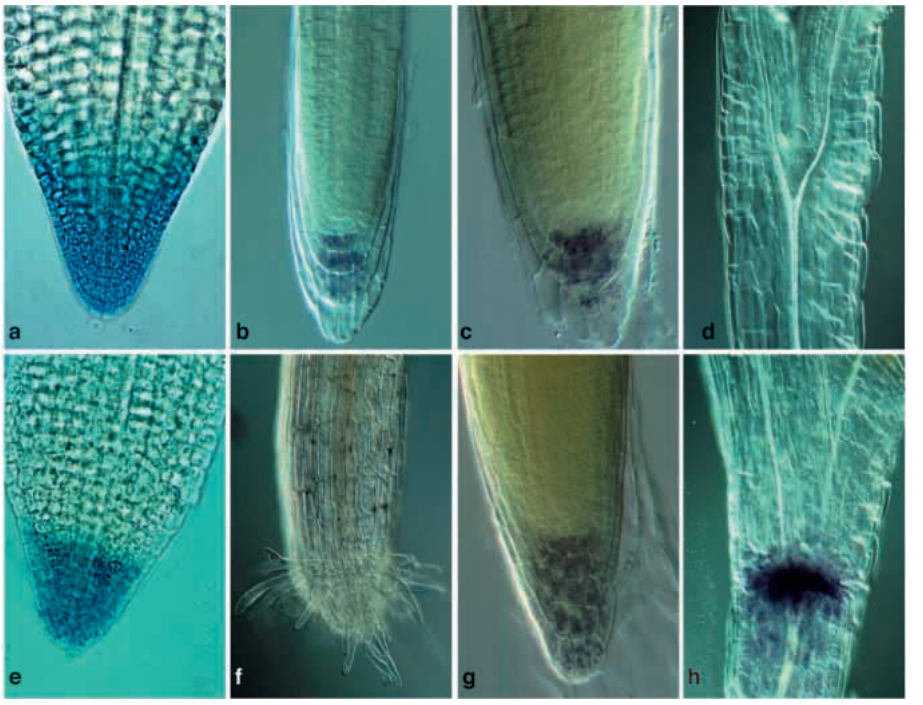

Fig. 3. Basal-marker staining in *bdl* mature embryos and seedlings. (a-d) Wild-type; (e-h) *bdl*. (a,e) Basal end of mature embryo showing root cap-specific GUS expression; note reduced domain of expression in *bdl*; (b,f) basal end of seedling: lugol staining of wild-type central root cap; no staining but root hairs in *bdl*; (c,g) postembryonic root tip: lugol staining of central root cap; (d,h) apical end of seedling; note lugol staining of shoot meristem region in *bdl* but not in wild-type.

These post-embryonic roots were initiated by periclinal cell divisions in the pericycle near the basal end of the axis (Fig. 2a,d). *bdl* post-embryonic root primordia were organised like wild-type lateral root primordia (Fig. 2b,e; cf. Malamy and Benfey, 1997) and eventually produced a root meristem at the distal tip (Fig. 2c,f). Thus, the *bdl* mutation only interfered with root formation in the context of embryogenesis.

To determine the alterations in *bdl* seedlings in more detail, we analysed the expression of root-cap markers (Fig. 3). Devic et al. (1995) had isolated a promoter trap line that shows expression of a GUS reporter gene in both the lateral and the central root cap of wild-type embryos and seedlings. The domain of GUS expression was reduced in *bdl* as compared to wild-type (Fig. 3a,e). Starch accumulation in the central root cap can be visualised by lugol staining (Van den Berg et al., 1997; Willemsen et al., 1998). No starch-specific staining was observed at the basal end of *bdl* seedlings, suggesting that the central root cap was missing although root hairs were formed (Fig. 3b,f). By contrast, lugol staining was observed in the tips of post-embryonically formed roots of *bdl* seedlings as in lateral root tips of wild-type seedlings (Fig. 3c,g). In contrast to wild-type, however, lugol staining was observed in the region of the *bdl* shoot meristem (Fig. 3d,h). We also noticed this ectopic accumulation of starch in *mp* seedlings (data not shown). Although the significance of this feature is not clear, the two mutants have in common a vascular defect which may impede sucrose transport at the base of the cotyledons, resulting in conversion of sucrose into starch.

The phenotypic similarity of *bdl* and *mp* seedlings raised the possibility that the *bdl* phenotype might be related to alterations in the response to auxin. We used the *auxin resistant1* (*axr1*; Lincoln et al., 1990) mutant as a control in experiments to define auxin-related phenotypes of *bdl* seedlings. Dark-grown *axr1* seedlings do not form the apical hook characteristic of wild-type seedlings in the dark, and the same phenotype was observed for *bdl* (Fig. 1b). Wild-type seedlings grown in the presence of the auxin analogue 2,4-D showed a swollen hypocotyl whereas *bdl* and *axr1* seedlings were not or only slightly affected, respectively (Fig. 1c). In addition, both *axr1* and *bdl* did not form callus on callus-inducing medium that readily transformed wild-type seedlings into callus (Fig. 1d). Thus, the *bdl* mutation interfered with auxin-mediated responses.

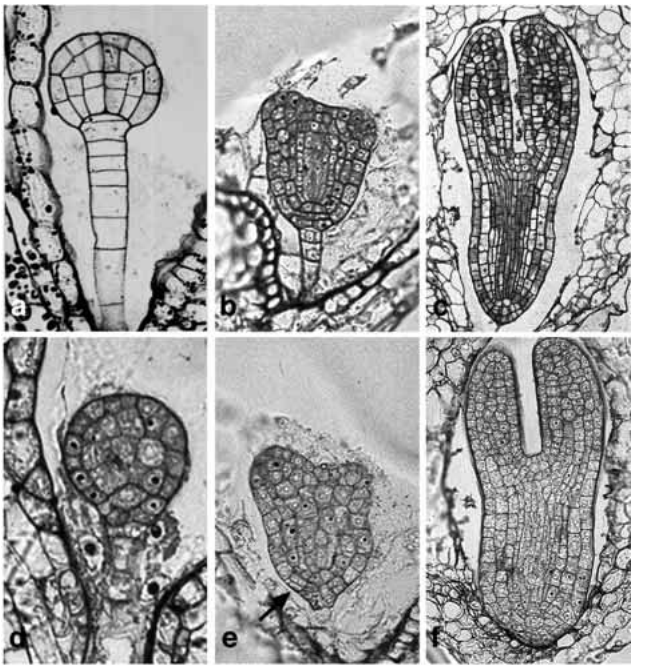

Fig. 4. Histological sections showing embryonic development of *bdl* primary root phenotype. (a-c) Wild-type; (d-f) *bdl*. (a,d) Mid-globular, (b,e) heart and (c,f) mid-torpedo stages. Note periclinal division at basal end of *bdl* heart-stage embryo (black arrow in e).

Table 2. Frequency of abnormal embryos in ovules from *bdl/BDL* plants

Parental genotype	2-8 cell stage	Globular stage	Heart stage	Torpedo stage	Total	% abnormal embryos
bdl/BDL	20/117	17/115	22/108	17/84	76/424	18
L*er* (control)	7/105	7/301	0/98	2/231	16/735	2

Development of *bdl* embryos

To determine the developmental origin of the primary root defect, we analysed successively younger stages of *bdl* embryogenesis (Fig. 4). From the globular to the torpedo stages, the cell group at the basal end, which normally displays the characteristic arrangement of hypophyseal cell derivatives (Scheres et al., 1994), was consistently abnormal. In addition, the cells in the vascular primordium lacked the prominent elongated cell shape in globular and heart stages but appeared nearly normal at the torpedo stage (compare Fig. 4d,e with 4f). It was of interest to note that *bdl* heart-stage embryos showed a periclinal division in the outermost cell layer just above the abnormal cell group at the basal end (Fig. 4e). This division, which normally generates an outer layer of lateral root cap cells and an inner layer of root epidermis cells, has been taken as an indication for the onset of root meristem activity (Scheres et al., 1994). In an alternative interpretation, this division could reflect radial patterning (see Discussion).

To trace the *bdl* defect back to the earliest stage of embryogenesis at which a deviation from wild-type development could be reliably observed, we quantitatively analysed whole-mount preparations of developing ovules from heterozygous *bdl/BDL* plants (Fig. 5; Table 2). In 18% of the ovules, the apical daughter cell of the zygote divided abnormally, i.e. horizontally instead of vertically, in contrast to 6% in L*er* wild-type (Fig. 5a,e,i; Table 2), which was reported previously to show some variability (Willemsen et al., 1998). From the octant stage, two modes of cell division patterns (type 1 and type 2) were consistently observed which may be correlated with the weak and strong phenotypes of *bdl* seedlings (see Fig. 1a). Type-1 octant-stage embryos consisted of twice the normal number of cells (Fig. 5b,f). At the globular stage, type-1 embryos looked apical-basally enlarged (Fig. 5c,g), and the corresponding heart-stage embryos showed a defect at the basal end whereas the vascular primordium appeared normal (Fig. 5d,h; compare with Fig. 4e). Type-2 octant-stage embryos displayed a file of several cells (Fig. 5j, compare with b and f). At the globular stage, these embryos had a short file of embryonic cells basally appended to a normal-looking embryo (Fig. 5k, compare with c and g). At the heart stage, type-2 embryos displayed abnormal vascular cell shapes in addition to the basal defect seen in type-1 embryos (Fig. 5l; compare with d and h). Thus, the earliest defect was observed in the apical daughter cell of the zygote whereas later on, the uppermost derivative of the basal daughter cell and its progeny did not undergo the stereotyped division pattern that would normally result in the formation of the quiescent centre of the root meristem and the central root cap.

Genetic interactions of *bdl* with other mutants

To determine whether *bdl* affects only the basal end of the developing embryo or has a more general effect on the development of the apical-basal pattern, we analysed double mutants involving *fackel* (*fk*) or *monopteros* (*mp*) (Fig. 6, Table 3). In *fk* embryos, hypocotyl development appears to be abnormal such that mutant seedlings have malformed cotyledons directly attached to the root (Mayer et al., 1991). *bdl fk* seedlings largely consisted of malformed cotyledons as evidenced by the characteristic shape of epidermal pavement cells and randomly distributed stomata (Fig. 6g,k,r). The basal end displayed a few root hairs as observed in the weak *bdl* seedling phenotype (Fig. 6s, compare with Fig. 6m). Thus, the double mutant phenotype suggests an additive effect of the two single mutations. However, considering the somewhat variable hypocotyl defect of the *fk* phenotype, we cannot exclude the possibility that the two mutations have a synergistic effect on

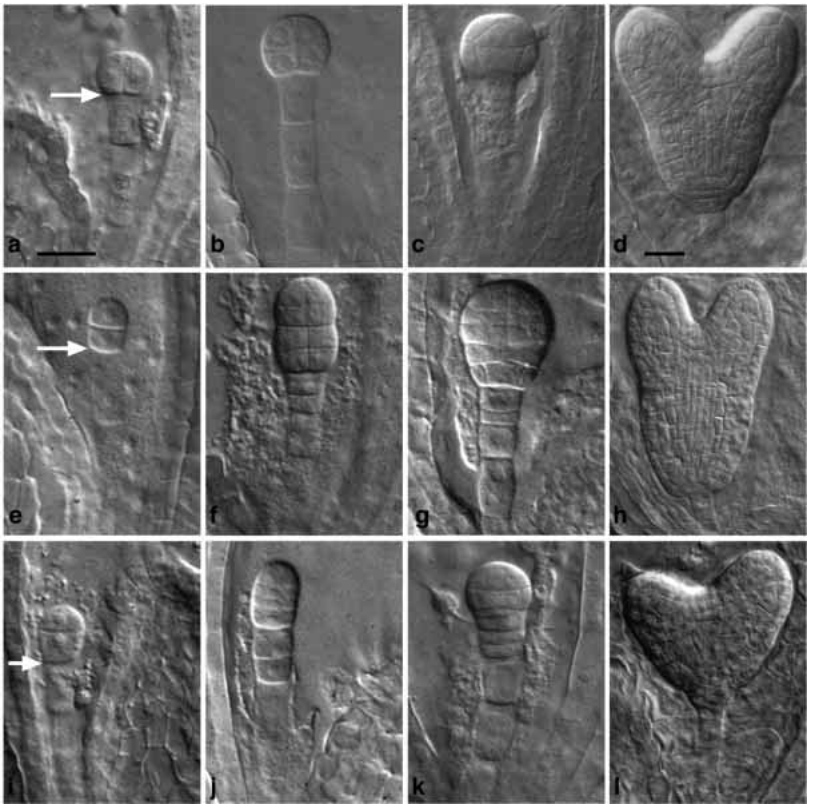

Fig. 5. Early stages of *bdl* embryogenesis. (a-d) Wild-type; (e-h) type-1 *bdl*; (i-l) type-2 *bdl* at the following stages: (a,e,i) 2-cell, (b,f,j) octant, (c,g,k) early/mid-globular; (d,h,l) early/mid-heart. White arrows mark the cell wall resulting from the division of the zygote in (a,e,i). Whole-mount preparations, Nomarski optics. Scale bars, 20 μm; (h,l) same scale as d; all others same scale as a.

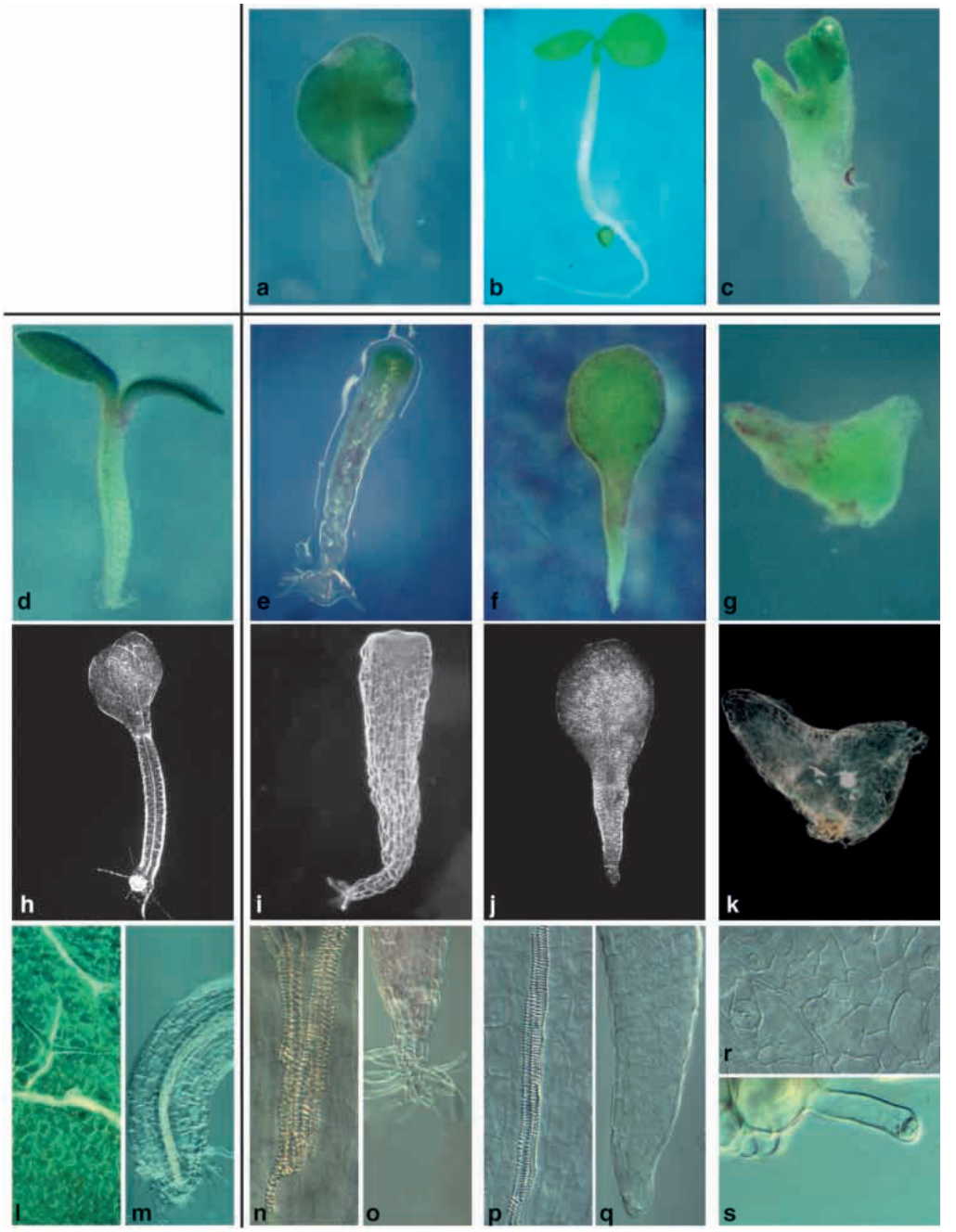

Fig. 6. Seedling phenotypes of double mutants. (a-d,h,l-m) Single mutants: (a) *mp*, (b) *axr1*, (c) *fk*, (d,h,l,m) *bdl*. (e-g,i-k,n-s) double mutants: (e,i,n,o) *bdl mp*, (f,j,p,q) *bdl axr1*, (g,k,r,s) *bdl fk*. (l) Vascular strands in rosette leaf with trichome. (m) Basal end of seedling with vascular strands and root hairs. (n) Vascular strands in hypocotyl. (o) Basal end of seedling with root hairs. (p) Vascular strands at base of cotyledon. (q) Basal end ('basal peg'). (r) Pavement cells and stomata. (s) Root hair at basal end of seedling. (a-g) Live seedlings. (h-k) Whole-mount preparations, dark-field optics. (l-s) Whole-mount preparations, Nomarski optics.

the apical-basal pattern. In *mp* embryos, the central and basal regions do not develop normally such that *mp* seedlings lack hypocotyl, root and root meristem, and the cotyledons are variably fused (Berleth and Jürgens, 1993). *bdl mp* seedlings displayed a new phenotype that was not equivalent to an additive effect of the two single mutations (Fig. 6e,i; compare with Fig. 6a,d,h). In addition to the absence of a root, these seedlings had strongly reduced cotyledons, thus essentially consisting of an elongated hypocotyl. The basal end of the seedling axis was marked by root hairs as are normally observed at the hypocotyl-root junction (Fig. 6o; compare with Fig. 6m). Although the cotyledons were barely distinguishable, the shoot meristem was present above the vascular strands (Fig. 6n) and formed primary leaves with trichomes (data not shown).

The embryonic origin of the *bdl mp* seedling phenotype was traced back to early stages. The earliest deviation was observed at the 2-cell stage when the apical daughter cell of the zygote had divided horizontally instead of vertically (Fig. 7a). This abnormality was very similar to the earliest abnormalities in the two single mutants, *bdl* (see Fig. 5e,i) and *mp* (Fig. 7f), which were essentially indistinguishable. At the octant/dermatogen stage, *bdl mp* embryos (Fig. 7b) resembled both *bdl* (see Fig. 5f) and *mp* (Fig. 7g), and the same was true for the globular stage (Fig. 7c; compare with Fig. 5g and Berleth and Jürgens, 1993). However, the *bdl mp* heart-stage embryo deviated from both *bdl* and *mp* embryos by the absence of bulging cotyledonary primordia and a widening of the upper end of the suspensor (Fig. 7d). The *bdl mp* torpedo-stage

Table 3. Frequency of *bdl* double mutants

(A) During embryogenesis

Parental genotype	2-8 cells	Globular	Heart	Torpedo	Total	% double mutants
bdl mp/BDL MP	5/32	34/168	15/65	4/22	58/287	20
bdl axr1/BDL AXR1	n.d.	8/39	29/78	46/194	83/311	27

n.d., not determined.

(B) At the seedling stage

Parental genotype	Total no. of seedlings	*bdl* weak phenotype	Other mutant phenotype	Double mutant phenotype	% double mutants
bdl MP/BDL mp	3,066	420	773 (*mp*)	140	5
bdl axr1/BDL AXR1	303*	14	18 (*axr1*)	32	11
bdl/ BDL; *fk/FK*	539	71	76 (*fk*)	17	3

*Germinated on 2,4-D.

embryo appeared wedge-shaped, which presaged the seedling phenotype (Fig. 7e; compare with Fig. 6e,i). Thus, although the earliest deviation was essentially the same in single- and double-mutant embryos, subsequent development of double-mutant embryos differed from the two single mutants.

In view of the auxin insensitivity of *bdl* seedlings, we also generated *bdl axr1* double mutants and analysed their phenotype (Table 3). The *bdl axr1* seedlings differed from *bdl* seedlings and rather resembled *mp* seedlings, showing a basal peg and variably fused cotyledons (Fig. 6f,j,p,q). When analysed during embryogenesis, differences between *bdl axr1* and *bdl* were only consistently observed from the globular stage. Double mutant embryos were markedly wider than *bdl* embryos as if their apical-basal axis was compressed (Fig. 7h; compare with Fig. 5g,k). At later stages, this compression of the axis became more pronounced such that the developing cotyledonary primordia accounted for most of the embryo (Fig. 7i,j; compare with Fig. 5d,h,l). The torpedo-stage embryo thus resembled a *mp* embryo with two cotyledonary primordia (see Berleth and Jürgens, 1993).

DISCUSSION

We have isolated a new mutant of *Arabidopsis* with very specific features that link the formation of the primary root meristem to auxin-mediated processes of apical-basal patterning in the embryo. The *bdl* mutation affects root formation in the context of embryogenesis but not root formation during post-embryonic development. The earliest defect observed in embryogenesis is an abnormally oriented division plane of the apical daughter cell of the zygote whereas later on the uppermost derivative of the basal daughter cell of the zygote fails to give rise to the quiescent centre of the root meristem and the central root cap. *bdl* seedlings are insensitive to the auxin analogue 2,4-D, and *bdl* adult plants have reduced apical dominance. They are short and bushy with rolled-up leaves. Furthermore, *bdl axr1* double mutants resemble *mp*, which shows reduced polar transport of auxin, and the initial embryonic phenotypes of *bdl* and *mp* are very similar, if not identical. We will first discuss the implications of the *bdl* early embryonic phenotype for the origin of the primary root meristem and then address how auxin may be linked to apical-basal patterning in embryogenesis.

The *bdl* early embryonic phenotype and the origin of the primary root meristem

The origin of the primary root meristem is currently viewed as the outcome of a signaling process between the progeny of the apical daughter cell of the zygote and the uppermost derivative of the basal daughter cell, with the latter attaining an extra-embryonic suspensor fate in the absence of a signal from the proembryo (for review, see Laux and Jürgens, 1997; Mayer and Jürgens, 1998). In early embryogenesis, the receiving cell becomes the hypophysis, or founder of the basal region, which

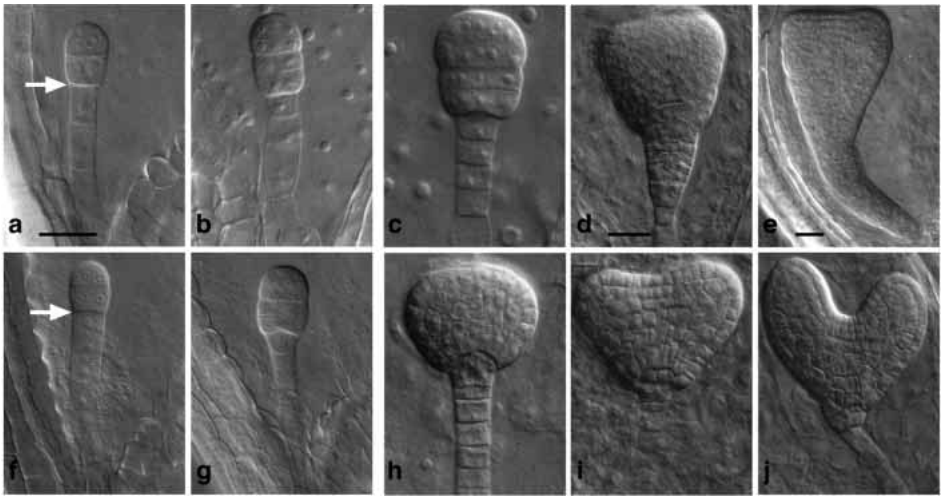

Fig. 7. Embryogenesis of *bdl mp* and *bdl axr1* double mutants. (a-e) *bdl mp*: (a) 2-cell with 4 nuclei, (b) octant, (c) early-globular, (d) heart, (e) torpedo stages. (f-g) *mp*: (f) 2-cell with 4 nuclei, (g) octant/dermatogen stages. (h-j) *bdl axr1*: (h) late-globular, (i) heart, (j) torpedo stages. White arrows mark the cell wall resulting from the division of the zygote in a,f. Whole-mount preparations, Nomarski optics. Scale bars, 20 μm; (a-c,f-h), d,i and e,j same scale.

gives rise to the quiescent centre of the root meristem and the central root cap (Scheres et al., 1994). The quiescent centre presumably signals back to the adjacent cells of the central region derived from the apical daughter cell of the zygote and maintains their fate as stem cells of the root meristem (Van den Berg et al., 1997). In this process, signaling back and forth across the clonal boundary between progenies of the apical and basal daughter cells of the zygote is instrumental in establishing the primary root meristem (Fig. 8). Support for this notion largely comes from the phenotypes of *hbt* and *mp* embryos. In *hbt* embryos, a progenitor of the hypophysis divides vertically instead of horizontally at the 2- or 4-cell stage of embryogenesis; subsequently, the basal region of the embryo is not established and no functional root meristem develops (Willemsen et al., 1998). In *mp* embryos, the apical daughter cell of the zygote gives rise to four rather than two tiers of embryonic cells at the octant stage whereas the basal daughter cell produces a normal file of cells (Berleth and Jürgens, 1993). Subsequently, the cell in place of the hypophysis divides like a suspensor cell and the cells in the central region of the embryo divide randomly, resulting in a failure of the embryo to produce hypocotyl, root and root meristem. Thus, *hbt* and *mp* appear to affect adjacent cells in the early embryo, suggesting cell-cell signaling in the formation of the hypophysis. We have now traced the *mp* defect to an even earlier stage. At the 2-cell stage, both *bdl* and *mp* affect the apical, but not the basal, daughter cell of the zygote, and its horizontal division results in a double-octant pro-embryo. Especially the weak (type 1) phenotype of *bdl* embryos only deviates from normal at the junction to the suspensor, lacking the quiescent centre, the central root cap and a primary root meristem (see Fig. 5h). The strong (type 2) phenotype of *bdl* embryos may be interpreted as a more pronounced defect: the lower daughter of the apical cell fails to change the plane of division from horizontal to vertical as did the apical cell itself, resulting in a failure to produce the derivatives of the central region, hypocotyl and root. Taken together, the *bdl* early embryo defect supports the notion that in normal development, the uppermost derivative of the basal daughter cell of the zygote switches fate from extra-embryonic to embryonic in response to a signal emanating from the apical cell or its progeny.

What is the evidence for another signaling event in primary root meristem formation, from the quiescent centre to the adjacent cells of the central region, at a later stage of embryogenesis (Fig. 8)? Although their earliest defects are different, both *hbt* and *bdl* embryos fail to establish a functional primary root meristem. Whereas *hbt* seedlings display the same defect during lateral root development (Willemsen et al., 1998), *bdl* seedlings form functional roots, including the distal root meristem, post-embryonically. Evidence from cell ablation studies at the seedling stage suggests that quiescent centre cells maintain the stem cell fate of adjacent root meristem initials (Van den Berg et al., 1997). By analogy, one might presume that in advanced-stage embryos, a signal from the quiescent centre prevents differentiation of the adjacent cell tier, thus establishing the root meristem initials. Taken together, the early and the late phenotypes of *bdl* embryos support the idea that two signaling events between two adjacent but clonally distinct cell populations are involved in generating the primary root meristem.

Although both *bdl* and *hbt* embryos lack a functional root meristem, the two mutants appear to differ in the establishment of a lateral root cap. *hbt* embryos only occasionally undergo a skewed division to give lateral root cap cells and do not express a lateral root cap marker in strong alleles (Willemsen et al., 1998). By contrast, *bdl* late-heart stage embryos display periclinal cell divisions at the basal end of the axis (see Fig. 4e). These divisions may generate a lateral root cap, as suggested by the staining pattern of the root cap-specific GUS marker. Although the *bdl* mutation may be caused by one particular allele, the absence of a functional primary root meristem raises the following question: does the lateral root cap cell layer originate from an epidermal tissue layer by a radial patterning event that depends on the apical-basal position of the cell tier rather than the activity of a functional root meristem? A meristem-independent origin of the lateral root cap would be consistent with results of cell ablation studies at the seedling stage which indicate that the existing radial pattern of the embryo conveys tissue-specific information to new cell tiers formed by the active root meristem (Van den Berg et al., 1995). However, more experiments are needed to clarify this issue.

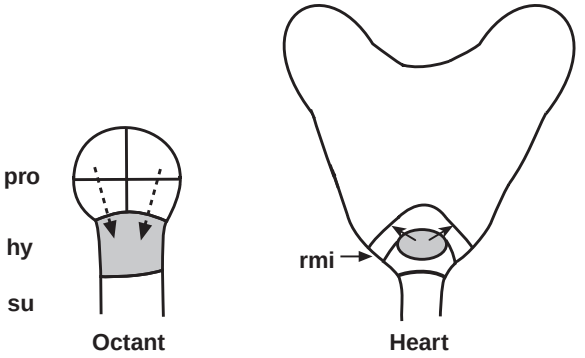

Fig. 8. A model for establishing the primary root meristem during embryogenesis. At the octant stage (left) a signal emanating from the progeny (pro) of the apical daughter cell of the zygote changes the cell fate of the uppermost derivative of the basal daughter cell (shaded) to become the hypophysis (hy), or progenitor of the quiescent centre and the central root cap. su, suspensor. At the heart stage (right) a signal from the quiescent centre (shaded) blocks differentiation of adjacent cells derived from the apical daughter cell of the zygote, thus conferring the fate of root meristem initials (rmi). For details, see text.

Auxin insensitivity of *bdl* and apical-basal patterning

By phenotypic criteria, the *bdl* mutation interferes with auxin response very much like mutations in the well-characterised *AXR1* gene (Lincoln et al., 1990; Leyser et al., 1993): no hypocotyl swelling, no apical hook in the dark, no callus formation (see Fig. 1). Unlike *bdl*, however, mutations in *AXR1* and *AXR3* (Rouse et al., 1998) alter the response to auxin without affecting apical-basal patterning in the embryo. Thus, the *bdl* phenotype combines two seemingly unrelated features, a defect in apical-basal patterning and insensitivity to the auxin analogue 2,4-D. Formally, two possible explanations can be distinguished. (1) There is no direct relationship between auxin and apical-basal patterning but the *bdl* mutation may affect patterning, with auxin insensitivity being an indirect consequence. (2) Alternatively, auxin may be directly involved in apical-basal patterning. Although the former possibility

cannot be ruled out at present, the available genetic and experimental evidence combined supports the latter.

Mutations in the *MP* gene, which encodes a transcription factor that appears to mediate auxin response (Hardtke and Berleth, 1998), and *bdl* give indistinguishable early-embryo phenotypes and similar seedling phenotypes. This suggests that *mp* and *bdl* mutations affect the same pathway. However, the seedling phenotype of the *bdl mp* double mutant points to a more complex genetic interaction: the cotyledon defect may be interpreted as synergy of the two mutations in the apical region whereas the presence of a hypocotyl suggests suppression of the *mp* phenotype by *bdl* in the central region. The double mutant phenotype also implies that *bdl* may affect the entire apical-basal pattern. In this context it is of interest to note that *bdl axr1* double mutants approach the *mp* seedling phenotype, suggesting that *axr1* enhances the apical-basal pattern defect of *bdl*. Furthermore, apical-basal pattern defects similar to the *mp* phenotype were mimicked by treatment of *Brassica juncea* embryos with auxin, auxin transport inhibitors and auxin antagonists (Hadfi et al., 1998). Along the same line, the cotyledon defect of *bdl mp* double mutants resembles the effect of auxin transport inhibitors in *Brassica juncea* embryos (Liu et al., 1993). Thus, the analysis of mutant phenotypes presented here and the results from experimental manipulation of embryos support the notion that auxin may play a role in apical-basal patterning during embryogenesis and that *bdl* may affect some aspect of this process. In this view, the same very early defect in embryogenesis of both *mp* and *bdl* raises the question of whether auxin signaling may come into play much earlier than previously shown by experimental analysis, due to the technical problems of manipulating embryos before the globular stage. The auxin insensitivity of *bdl* suggests that the abnormal orientation of the division plane at the two-cell stage may result from altered auxin perception, transport or response. However, it is entirely unclear where auxin is produced, how it is transported and perceived at this early stage of embryogenesis and how auxin may effect a reorientation of the cell division plane. In view of the technical difficulties, a molecular analysis of the *BDL* gene may be a starting point to address this issue.

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