Development 117, 149-162 (1993)
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Apical-basal pattern formation in the *Arabidopsis* embryo: studies on the role of the *gnom* gene

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SUMMARY

***gnom* is one of several genes that make substantial contributions to pattern formation along the apical-basal axis of polarity in the *Arabidopsis* embryo as indicated by the mutant seedling phenotype. The apical and basal end regions of the body pattern, which include the meristems of the shoot and the root, fail to form, and a minority of mutant embryos lack morphological features of apical-basal polarity. We have investigated the developmental basis of the *gnom* mutant phenotype, taking advantage of a large number of EMS-induced mutant alleles. The seedling phenotype has been traced back to the early embryo in which the asymmetric division of the zygote is altered, now producing two nearly equal-sized cells. The apical daughter cell then undergoes abnormal divisions, resulting in an octant embryo with about twice the normal number of cells while the uppermost derivative of the basal cell fails to become the hypophysis, which normally contributes to root development. Consistent with this early effect, *gnom* appears to be epistatic to *monopteros* in doubly mutant embryos, suggesting that, without prior *gnom* activity, the *monopteros* gene cannot promote root and hypocotyl development. On the other hand, when root formation was induced in bisected seedlings, wild-type responded whereas *gnom* mutants failed to produce a root but formed callus instead. These results suggest that *gnom* activity promotes asymmetric cell division which we believe is necessary both for apical-basal pattern formation in the early embryo and for root formation in tissue culture.**

Key words: *Arabidopsis*, embryo, apical-basal pattern, polarity, *gnom* gene

INTRODUCTION

In flowering plants, embryogenesis generates the primary body organisation as represented by the seedling to which new structures are added from the meristems of the shoot and the root during postembryonic development. The meristems arise as terminal elements of the apical-basal pattern along the axis of polarity. Additional elements are cotyledon(s), hypocotyl and radicle (embryonic root) which do not contribute to postembryonic development. The main tissue types such as epidermis, ground tissue and vascular tissue form a second pattern in the seedling which is organised perpendicular to the axis of polarity.

One approach to studying how the body organisation is established in the plant embryo makes use of mutants that interfere with embryogenesis. Embryonic-lethal mutants, which have been isolated both in *Arabidopsis* and in maize, are being characterised genetically, developmentally and in tissue-culture experiments (Müller, 1963; Meinke and Sussex, 1979; Meinke, 1985; Clark and Sheridan, 1991; review by Meinke, 1991). We have recently isolated and characterised mutants of *Arabidopsis* in which different aspects of the seedling organisation are altered (Mayer et al., 1991). For example, mutations in four genes delete different parts of the apical-basal pattern, and these seedling phenotypes were traced back to specific defects in the developing mutant embryos. The mutant phenotypes suggested that the apical-basal axis is partitioned into three regions, roughly corresponding to epicotyl and cotyledons, hypocotyl and root. Mutations in the *gnom* gene produced a minority of seedlings lacking morphological features of apical-basal polarity. Since this polarity is normally correlated with the asymmetric division of the zygote, we have performed a detailed genetic and phenotypic analysis of the *gnom* gene to determine its role in apical-basal pattern formation. Our results suggest that the *gnom* gene very likely acts before the asymmetric division of the zygote when apical-basal polarity first becomes visible, and that the *gnom* gene is also required for root formation in tissue culture.

MATERIALS AND METHODS

Plant growth conditions

Seeds were sown on wet soil (sterilised mixture of 1 part potting

soil, 1 part sand) in 5 cm square plastic pots. The pots were covered with plastic bags, stored in the cold room for 4 days to break dormancy, and then transferred to a constant-temperature room (set at 24°C unless otherwise stated) with continuous illumination (Philips W 84 light bulbs, about 6.000 lux at the level of the pot) and 70% humidity. Three days later, the plastic bags were removed, and the seedlings were initially sprayed with water and later watered daily from below.

Plant strains

The wild-type used was the Landsberg *erecta* (*Ler*) ecotype. Mapping experiments involved the marker strains W100 (*an ap-1; er py; hy-2 gl-1; bp cer-2; ms-1 tt-3*), W2 (*an dis-1*) and W3 (*ga-4 dis-2 cer-5*) kindly provided by M. Koornneef, Agricultural University Wageningen, The Netherlands (for description of mutants, see Koornneef et al., 1983). The *gnom* (*gn*) mutants and the *monopteros* (*mp*) allele *U55* were isolated on the basis of their seedling phenotypes following EMS mutagenesis of *Ler* seeds (Mayer et al., 1991). The fused-cotyledon mutant *emb30* (originally named 112A-22; Meinke, 1985; Patton and Meinke, 1990) was kindly provided by D. W. Meinke, Oklahoma State University, Stillwater, OK, USA.

Genetic crosses

Flowers of plants to be used as female parents were emasculated by removing the anthers with forceps. These flowers were pollinated the following day by touching the stigma with anthers from the male parent. Generally, 2-4 successive flowers of the same female plant were used in the same cross.

Complementation tests

Plants to be used as parents were first tested for *gnom* heterozygosity by phenotypic analysis of late embryos from selfed flowers. Younger flowers of heterozygous plants were then used for test crosses. F_1 seedling progenies from at least 3 pollinated flowers were phenotyped on agar plates for each complementation test.

Recombination mapping

Flowers from *gnom* heterozygous plants were used to pollinate homozygous marker plants. Among the F_1, *gnom* heterozygous plants were identified by the phenotypes of embryonic progeny. Individual F_2 plants were phenotyped for markers and tested for the segregation of *gnom* seedlings (F_3 progeny) on agar plates. Map distances were calculated from recombination frequencies (RF) using the mapping function. RF values were determined as $RF=\mathbf{x}/(2-\mathbf{x})$ where **x** is the proportion of *gnom* heterozygous among all F_2 plants showing the same marker phenotype. As a control, the RF value for *an - dis-1* was calculated: $\mathbf{x}=(1-RF)^2$ where **x** is the proportion of *an dis-1* among all F_2 plants showing the *an* (or *dis-1*) phenotype.

Construction of *gn mp* double mutant

Trans-heterozygous *gn* +/+ *mp* F_1 plants, which had been selected from a cross of $gn^{4\text{-}13}$/+ × mp^{U55}/+, were selfed to produce a large number of F_2 plants among which putative cis-heterozygous *gn mp*/+ + plants were identified by progeny testing (see Table 4a). The *gn mp*/++ genotype of one F_2 plant was verified by raising and genotyping F_3 plants (see Table 4b) and by back-crossing with mp^{U55}/+ (see Table 4c).

Analysis of seedling phenotypes

Seeds were plated on 0.3% agar plates. The plates were incubated at 4°C for 4 days and then exposed to light at 24°C for 4 days. Photographs were taken with an Olympus Ti-4 camera attached to an Olympus stereoscope, using a Kodacolor 100 Gold film.

Whole-mount preparations

Embryos or seedlings were fixed in 6:1 ethanol/acetic acid and mounted in freshly prepared 8:3 chloralhydrate/water. Phenotypes were analysed with a Zeiss Axiophot using phase-contrast or Nomarski (DIC) optics. Photographs were taken using an Agfa Pan 25 film.

Histological analysis

Siliques of appropriate stages were removed, attached to double-sided Scotch tape and slit open with an injection needle. Ovules were transferred to fixative (4% formaldehyde, 0.25% glutaraldehyde, 50 mM sodium phosphate buffer pH 7.2) and fixed overnight at 4°C. After washing with buffer and water, the ovules were dehydrated and then infiltrated with 7:1 acetone / TAAB resin (TAAB Company) overnight. Several changes of TAAB's solution of increasing concentrations (5:1, 3:1, 1:1; 1:3, 1:7) were carried out for 3-4 hours, then 3 changes of 100% TAAB's solution in which the ovules were left overnight. Hardening was done at 60-80°C for at least 8 hours. Serial sections (3-5 μm thick) were cut with a Reichert-Jung microtome, transferred to polylysine-coated microscopic slides, stained with 0.05% toluidine-blue at 60°C for 20 seconds and embedded in TAAB's. The sections were analysed with a Zeiss Axiophot microscope using bright-field illumination with a yellow filter. Photographs were taken using an Agfa Pan 25 film.

Tissue culture

Seeds were surface-sterilised by shaking in commercial bleach (Klorix) for 20 minutes and rinsing with 70% ethanol. Seeds were extensively washed with water and transferred to 0.3% agar plates for germination. Intact seedlings or seedlings bisected with an injection needle were transferred to plastic Petri dishes containing agar-solidified root-inducing medium which consisted of 50% msv mix (Murashige-Skoog basal medium and vitamins, purchased from Sigma, catalogue no. M0404; Murashige and Skoog, 1963), 0.5% sucrose, 0.7% agar, 50 μg/ml ampicillin and 3 μg/ml indole butyric acid (IBA).

RESULTS

The *gnom* seedling phenotype is caused by mutations in a single gene

The *gnom* seedling phenotype represents one of four apical-basal pattern deletion types, with both apical and basal structures being absent, and a minority of mutant seedlings lack morphological features of apical-basal polarity. This heterogeneous phenotype, which was originally described for each of 15 lines carrying allelic mutations (Mayer et al., 1991), has also been observed in 9 additional mutant lines (Fig. 1). To understand the genetic basis of the *gnom* phenotype, all 24 mutant lines were subjected to complementation analysis (Table 1). Most of the 276 possible pair-wise combinations of mutants were generated and about a quarter of them were also tested in reciprocal crosses. Each combination produced *gnom* seedlings that roughly amounted to 25% of the progeny (Table 1). Thus, the *gnom* phenotype is caused by recessive mutations in a single, zygotically active gene.

Most trans-heterozygous combinations gn^x/gn^y produced essentially the same range of mutant seedling phenotypes as each of the homozygous parental alleles (Fig. 1B-J,L; see below). However, certain allele combinations yielded a

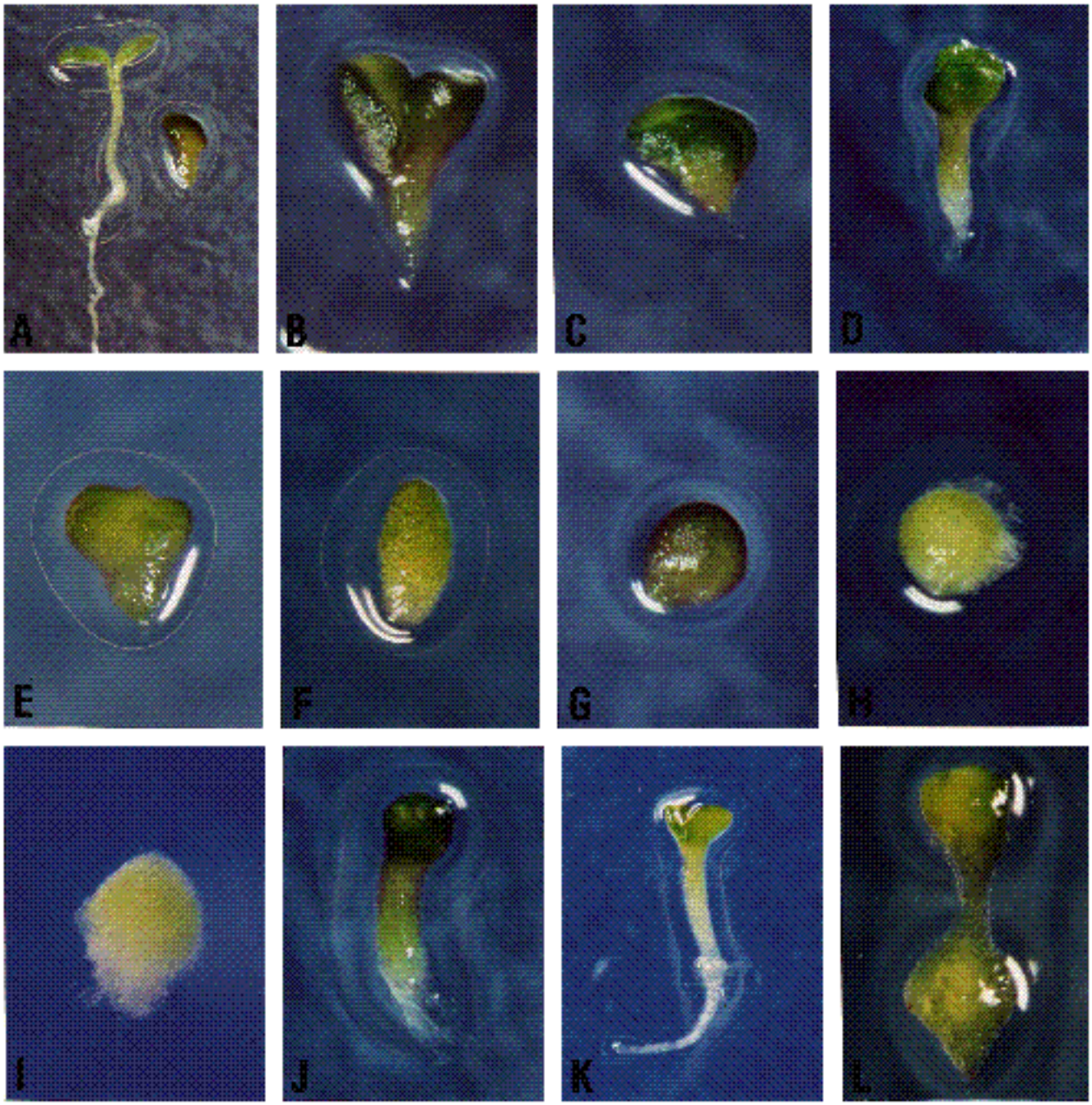

Fig. 1. Phenotypes of *gnom* mutant seedlings. (A) Wild-type seedling on the left and 'cone-shaped' *gnom* mutant seedling on the right; (B-D) 'cone-shaped'; (E,F) 'oblong'; (G,H) 'ball-shaped'; (I-K) 'root' phenotypes: (I) 'oblong' type, (J) 'cone-shaped', (K) 'weak' phenotype of $gn^{4\text{-}13}/gn^{U87}$ trans-heterozygote; (L) 'double *gnom*'.

novel phenotype that can be described as weak because it was intermediate between the wild-type pattern and the regular *gnom* phenotype (Fig. 1K). Depending on the allele combination, 10-82% of the *gnom* seedlings showed this weak phenotype. By this phenotypic criterion, the *gnom* alleles fall into 3 groups which we name A, B and C. Trans-heterozygotes for mutants within each group as well as combinations between C and A or B mutants showed the strong phenotype whereas trans-heterozygotes for A and B mutants gave the weak phenotype (Table 1). While the majority of alleles represent group A, only two alleles, T391 and U87, belong in group B and two other alleles, S28 and U207, are members of group C (Table 1). We have recently obtained the *emb30* (=112A-2A) mutant which had been described by Meinke (1985) as a late embryonic-lethal mutant with a 'fused-cotyledon' phenotype, and a few test crosses indicated that *emb30* is a *gnom* allele which belongs in either group A or C (data not shown; see Table 3). The complementation data thus enable distinctions to be made between group A, B and C alleles which all produce the

Table 1. Complementation analysis of *gnom* mutants

G60	20/29	X																				
R48	19/22	19	X																			
R304	16/21	33/25	26	X																		
R310	20/20	43/22	22	33/20	X																	
T97	nc	ND	21	23/26	17/20	X																
T339	24/25	24/38	24	ND	19/25	31	X															
T345	30	36/28	28	20	32	ND	22	X														
T424	nc	26	ND	20	28	ND	ND	ND	X													
U23	16/24	21/40	18	19/28	21	25	34/21	28	ND	X												
U40	25/14	20/39	31/26	22	22	26	27	ND	ND	22/18	X											
U75	20/31	19/28	26	35/24	37	35	20/37	17	nc	24	24	X										
U147	13/25	37/20	26	20	20	nc	20	nc	23	13	26	10	X									
U221	27/21	14/27	25	20	23	18	ND	17	nc	16	19/43	20	28	X								
U223	11/20	39	ND	19/20	ND	ND	22	nc	ND	24	22	ND	25	ND	X							
U228	9/23	23/19	30	27	21/21	16	21	29	nc	nc	21	30/21	20	20	nc	X						
U243	19	11	20	21	18/37	22	ND	25	ND	27	20	13	29	30	ND	22/31	X					
U255	29/21	26	23	21/16	18/17	19	19	24/33	ND	29/33	25	26/23	32/29	24/22	30	24	27	X				
U263	20	33	13/27	24	20	23/16	23	26/23	15/20	22	22/20	23/23	26	21/15	26	14	23/19	25	X			
U323	25/20	34/23	nc	38/24	20/18	23	25	21	19	23/24	26/30	19/19	21	16/23	22	20/17	18	19/30	21	X		
T391	25/21*	45*	30*	17*	28*	19*	9	25*	22*	31*	22*	16*	26*	22/25*	ND	21*	19*	16/27*	19*	29/24*	X	
U87	14*	22	ND	ND	ND	8	ND	ND	ND	ND	25	17*	26*	15*	29*	ND	22	29	23*	24*	23⁻	X
S28	nc	ND	14/36	23	22/20	27	ND	27	ND	22/23	34/22	14	27	20/25	17/26	18	16	ND	26	ND	16⁻	22/26	X
U207	23/23	41	36	21	23	ND	26	24/22	ND	21	29	26	ND	32	ND	29/18	ND	28	ND	22	20⁻	20/19	23
	4-13	G60	R48	R304	R310	T97	T339	T345	T424	U23	U40	U75	U147	U221	U223	U228	U243	U255	U263	U323	T391	U87	S28

Allele designations are given on the left and at the bottom. Allele groups A, B and C are separated by double lines (see text). Numbers indicate percentages of *gnom* seedlings, with the total number of seedlings exceeding 50 and often 100. The results of reciprocal crosses are separated by slashes. nc (=non-complementing) signifies crosses yielding *gnom* seedlings but the total number of seedlings was either less than 50 or not determined. * marks the occurrence of 'weak phenotype' seedlings (see Fig. 1K); ⁻ indicates that no 'weak phenotype' seedlings were observed; ND, not determined.

same range of phenotypes, suggesting that the regular *gnom* phenotype results from complete gene inactivation (see Discussion).

Mapping of the *gnom* gene

The *gnom* gene was initially mapped by crossing the $gn^{4\text{-}13}$ allele with the W100 marker strain (see Materials and Methods). Most of the F_2 plants showing the *an* phenotype did not produce *gnom* seedlings, indicating close linkage of the two genes (data not shown). To map the *gnom* gene more precisely, a three-factor cross was done, involving the first-chromosome markers *an* and *dis-1* (Koornneef et al., 1983). Each of 558 F_2 plants from this cross was phenotyped for the recessive markers and then genotyped for *gn* by progeny-testing (Table 2). The rarest phenotypic classes were the reciprocal recombinants, *an* plants that did not produce *gnom* seedlings and *dis-1* plants that did produce them. Thus, the order of the 3 genes on the chromosome is *an*, *dis-1*, *gn*, separated by approximately 24 cM and 2 cM, respectively (for calculation of map distances, see Materials and Methods). This result is consistent with the mapping data for the *emb30* mutant (Patton et al., 1991). The map position of the *gn* gene was subsequently confirmed in another cross involving the first-chromosome markers *ga-4*, *dis-2* and *cer-5* (Koornneef et al., 1983), which placed *gnom* to the left of *ga-4* (data not shown). In summary, the *gnom* gene is located between *dis-1* and *ga-4* on the first chromosome.

All *gnom* alleles produce a range of different seedling phenotypes

Mutations in the *gnom* gene severely disrupt the apical-basal pattern of the seedling which in the wild-type consists of the following elements: shoot meristem, cotyledons,

Table 2. Recombination mapping of the *gnom* gene

F_2 marker phenotype	Total number of F_2 plants	*gn*/+	+/+
an dis-1	63	3[a]	60
an +	52	49[b]	3
+ *dis-1*	44	0[c]	44
+ +	399	389	10
∑ F_2	558	441	117

F_1 plants of the genotype *an dis-1* + / + + *gn* were selfed. Recombination occurs in both female and male meiosis so that the *gn*/+ F_2 plants showing the recessive marker phenotypes [a, b, c] are most suitable for determining the map position of the *gnom* gene. Since [b] > [c], *gnom* is closer to *dis-1* than to *an*, and since [a] > [c], *gnom* lies outside the *an-dis-1* interval, assuming that single crossovers are more frequent than double crossovers. If *gnom* were to lie between *an* and *dis-1*, this would result in [c] > [a].

[a]due to crossover between *dis-1* and *gn* (genotype: *an dis-1 gn* / *an dis-1* +).
[b]due to crossover between *an* and *dis-1* (genotype: *an* + *gn* / *an dis-1* +).
[c]due to double crossover (genotype: + *dis-1 gn* / *an dis-1* +) or crossovers in both female and male meiosis (genotype: *an dis-1 gn* / + *dis-1* +).

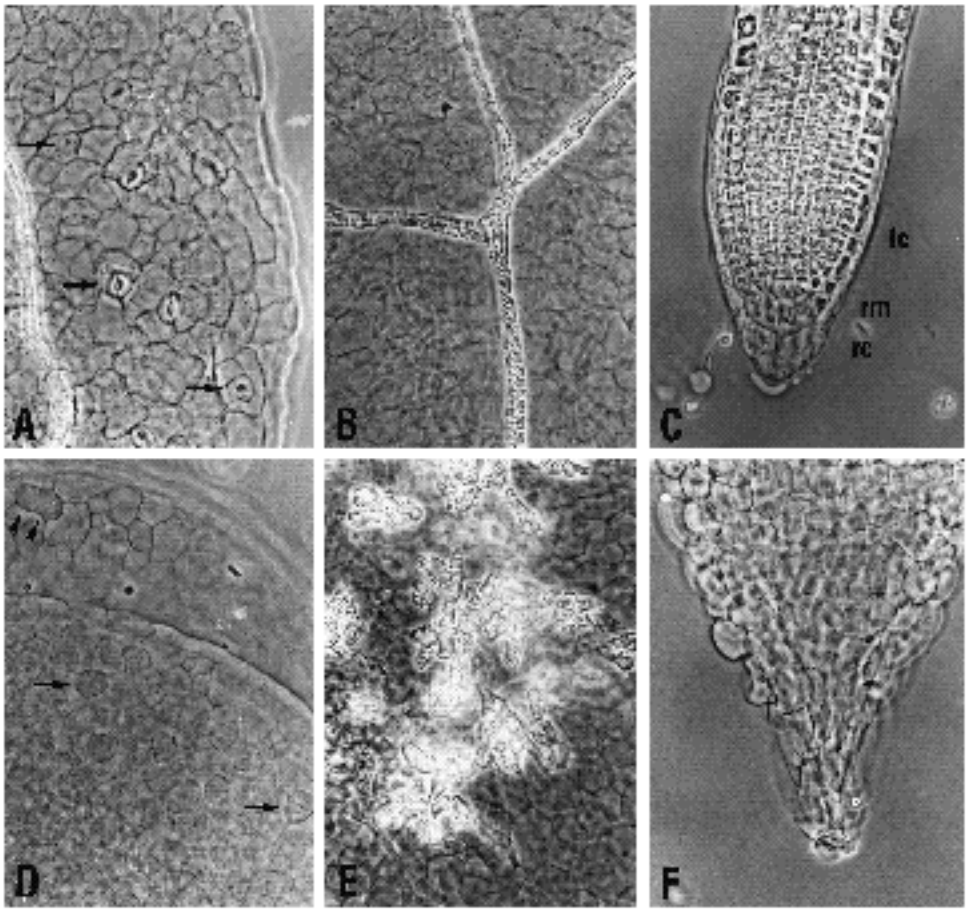

Fig. 2. Details of wild-type (A-C) and 'cone-shaped' *gnom* (D-F) seedlings. (A,D) Epidermis of cotyledon: stomata (arrows) are present and can be adjacent in the mutant (arrowheads). (B,E) Vascular tissue of cotyledon: vascular cells are not interconnected in the mutant. (C,F) Root end: the mutant lacks files of root cells (fc), root meristem (rm) and root cap (rc). Phase-contrast optics.

hypocotyl, embryonic root (radicle), root meristem and root cap (Figs 1A, 2A-C). All *gnom* alleles produced essentially the same range of mutant seedlings rather than distinct phenotypes representing different degrees of deviation from the normal pattern. To facilitate description, we have established phenotypic classes although in reality the mutant seedling population may represent a continuum of phenotypes (Fig. 1). The vast majority of mutant seedlings lacked any morphological sign of root differentiation, and this group accounted for 86-99%, depending on the *gnom* allele (Fig. 1B-G; Table 3).

Three major phenotypes were distinguished on the basis of shape and regional differentiation: 'ball-shaped', 'oblong' and 'cone-shaped'. In terms of apical-basal polarity, the 'ball-shaped' seedling represents the strongest phenotype: the seedling looks uniform, lacking cotyledons and root, and no apical-basal axis is apparent (Fig. 1G). The radial pattern of the 3 main tissue types is, however, formed although the centrally located vascular tissue consists mainly of single cells not interconnected to form strands (Mayer et al., 1991). That the ground tissue of the 'ball-shaped' seedlings may correspond to ground tissue of wild-type hypocotyl is suggested by the light green colour. We observed infrequent variants that were covered with hairs, with the 'ball' being light green (Fig. 1H) or white (not shown). The 'oblong' seedling has a similar uniform colouration of light green but its shape is different, varying from oblong to branched or other forms (Fig. 1E,F). Although the 'oblong' seedling lacks both cotyledons and root, it seems to have an axis as indicated not only by its shape but also by the distribution of vascular cells which are not localised as a compact group but scattered along the axis (not shown). By this criterion, the 'oblong' seedling represents a weaker phenotype than the 'ball-shaped' seedling. The third and most numerous class, the 'cone-shaped' seedling is an even weaker phenotype in that regional differentiation clearly indicates apical-basal polarity (Fig. 1B-D; Table 3). 'Cone-shaped' seedlings lack the root whereas the dark green cotyledons vary from nearly normal in shape (Fig. 1B) to strongly reduced and fused to form a funnel (Fig. 1C,D), and the light green hypocotyl may be reduced or elongate (Fig. 1D). When the cotyledons are clearly separate, abnormal leaves are formed, indicating that a shoot meristem was established in the embryo.

The other mutant seedling types can be regarded as rare variants of the major phenotypes. For example, 'double gnom' seedlings, which occurred at very low frequency, resemble two interconnected 'ball-shaped' seedlings (Fig. 1L), with the two globular masses containing vascular tissue in the centre (data not shown). We also observed variants of the 'oblong' and 'cone-shaped' seedlings that had root hairs at one end, and these variants were therefore included under 'root' phenotype (Fig. 1I,J; Table 3). However, the 'roots' of these seedlings were unable to grow, suggesting that they lacked a functional root meristem. In addition, root hairs are normally found at the basal end of the hypocotyl

Table 3. Seedling phenotypes of *gnom* alleles

Allele number	group*	Number of seedlings	% *gnom*	% of different *gnom* seedling phenotypes† 'cone'	'ball'	'oblong'	'root'‡	other§
4-13	A	1,728	20.3	84	1	9	3	
G60	A	2,628	38.6	92	1	6	1	
R48	A	1,340	23.6	91	2	5	3	<1
R304	A	1,837	21.4	91	3	4	3	
R310	A	1,035	21.9	91		7	1	
S28	C	637	30.5	91	1	7	1	1
T97	A	1,319	26.1	94	<1	1	4	
T339	ND	326	23.9	99			1	
T345	A	530	23.8	94	1	4	2	
T391	B	641	19.7	95	1	2	2	
T424	A	267	23.2	84		14	2	
U23	A	396	21.2	90	4	5	1	
U40	A	649	27.6	83	3	12	3	
U75	A	1,178	20.6	92	3	3	2	
U87	B	641	19.8	67	23	8	2	
U147	A	873	19.9	93	1	5	2	
U207	C	1,406	25.1	86	3	8	3	
U221	A	749	24.3	62	9	15	14	
U223	A	852	17.9	87	5	5	3	
U228	A	421	21.6	73		25	1	
U243	A	709	22.0	90	3	3	4	
U255	A	1,232	25.7	86	1	7	6	
U263	A	1,519	25.1	85	2	8	6	
U323	A	2,110	22.5	91		6	1	
emb30-1	ND	343	28.0	98		2		

*based on complementation data (Table 1; see text); ND, not determined.
†classification of phenotypes as in Fig. 1.
‡seedlings with hairs at basal end (see Fig. 1I,J); for details see text.
§mostly 'double gnom' phenotype (see Fig. 1L).

and in the root, and the root hairs of the freshly emerged seedling appear not to derive from the root meristem (B. Scheres, personal communication). While such 'root' phenotypes were infrequently observed among homozygous mutant seedlings, trans-heterozygous gn^A/gn^B seedlings regularly showed the weakest phenotype of all: a reduced embryonic root, with differentiated root hairs, was attached to an elongated hypocotyl (Fig. 1K, compare with Fig. 1A). Such seedlings were, however, unable to sustain root development on soil or when grown in tissue culture (see below). In constrast to the root defect, the cotyledons were variably affected showing the same range of changes as the 'cone-shaped' seedlings (compare Fig. 1K with B-D).

We analysed the region-specific differentiation of mutant seedlings at the cellular level, and the results for the 'cone-shaped' seedlings are shown in Fig. 2. The apical defect consists of strongly reduced cotyledons that can be recognised by the occurrence of stomata in the epidermis (Fig. 2A,D). In contrast to wild-type, stomata were sometimes found adjacent to one other in the mutant (Fig. 2D). Stomata were not observed in 'oblong' or 'ball-shaped' seedlings or in the hypocotyl of wild-type seedlings (data not shown). The vascular strands, which run down the length of the wild-type seedling, do not form properly in *gnom* mutant seedlings although vascular cells do differentiate. The vascular cells are loosely arranged in the cotyledonary region of 'cone-shaped' seedlings (Fig. 2B,E). The basal defect of the regular *gnom* phenotype corresponds to the absence of the root. In wild-type seedlings, 3 regions of the root can be distinguished: the embryonic root (radicle) which is characterised by files of cells derived from the root initials of the meristem, the root meristem organised around the quiescent centre, and the root cap which covers the root tip (Fig. 2C). None of these regions can be recognised by morphological criteria in 'cone-shaped' seedlings (Fig. 2F). The root meristem and the root cap are also absent in the weakest phenotype which is produced by gn^A/gn^B trans-heterozygotes (data not shown).

The regular *gnom* phenotype was rather variable, ranging from the lack of apical-basal polarity to mild apical defects and concomitant absence of the root end. This phenotypic spectrum was essentially the same for each allele although the alleles differed in their complementation behaviour. We were therefore interested to determine whether a different growth temperature of 18°C instead of 24°C would reveal phenotypic differences between the alleles. This was not the case. There were neither weaker phenotypes such as in the trans-heterozygotes nor only 'ball-shaped' seedlings, indicating that none of the alleles is markedly temperature-sensitive. Thus, the variability of the regular *gnom* phenotype, rather than the lack of apical-basal polarity, might represent the inactive state of the *gnom* gene (see Discussion), which raised the question of how the regular *gnom* phenotype originates in the embryo.

Development of *gnom* mutant embryos

The *gnom* seedling phenotype had been traced back to the heart-stage embryo which displays abnormalities at both the apical and the basal end (Mayer et al., 1991). We have therefore compared, in histological sections, the early stages of embryogenesis in wild-type and *gnom* mutant lines to determine when and how the mutant embryos begin to devi-

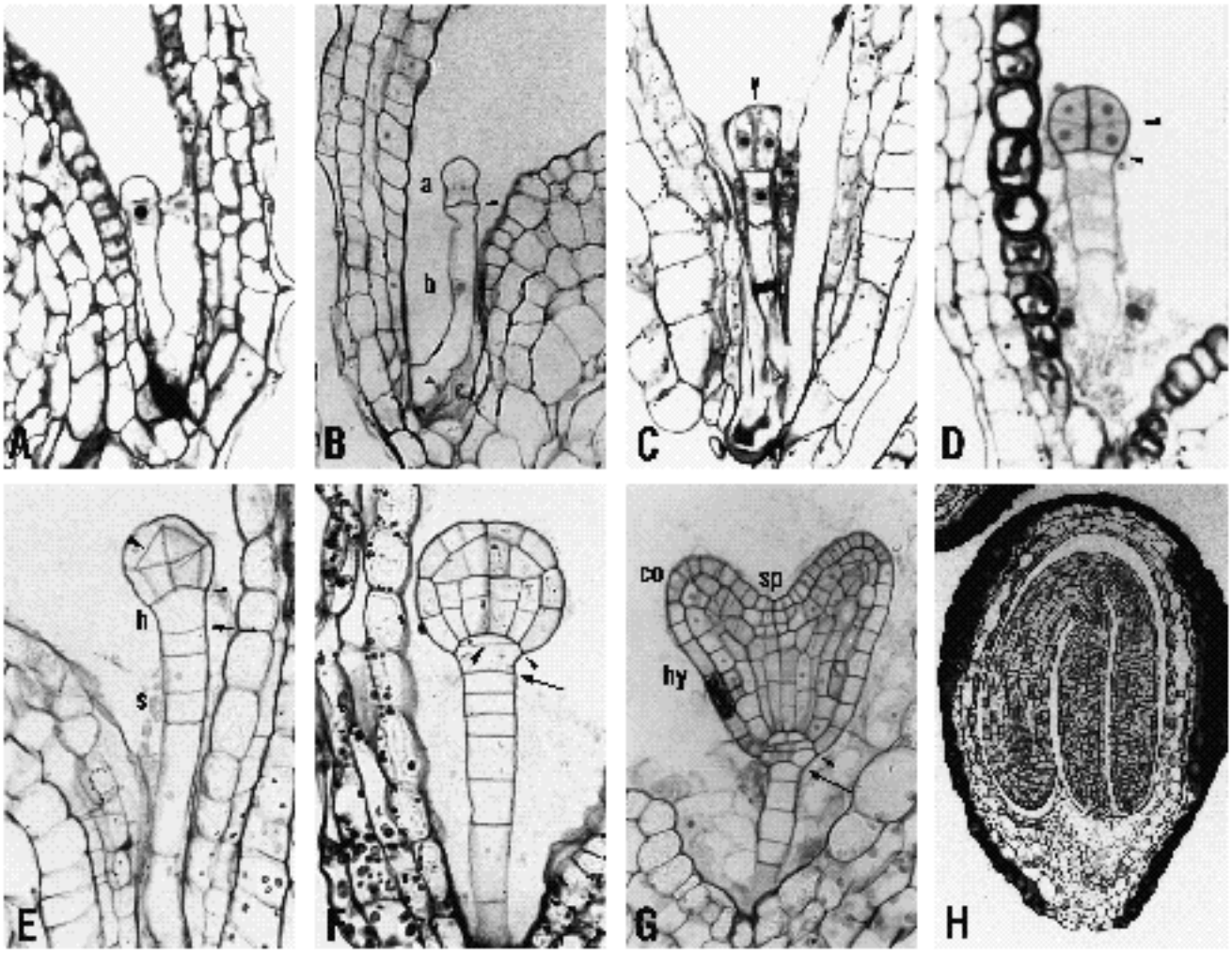

Fig. 3. Wild-type embryogenesis (stages after Jürgens and Mayer, 1992). (A) Elongated zygote (stage 2) with the nucleus in a sub-apical position. (B) One-cell stage (stage 3) showing the apical (a) and basal (b) daughter cells of the zygote. (C) Two-cell stage (stage 4) after vertical division of the apical cell (large arrowhead). (D) Octant stage (stage 6) derived from four-cell stage by horizontal divisions (large arrowhead). (E) Dermatogen stage (stage 7) showing outer layer of epidermal precursor cells produced by tangential divisions (large arrowhead); the uppermost derivative of the basal cell is the hypophysis (h), with the remaining derivatives forming the suspensor (s). (F) Mid-globular stage (stage 9): the hypophysis has divided asymmetrically (large arrowhead). (G) Mid-heart stage (stage 13) with the primordia of shoot meristem (sp), cotyledons (co) and hypocotyl (hy). (H) Mature-embryo stage (stage 20). Small arrowheads, in B-G, mark the cell wall produced by the asymmetric division of the zygote, arrows, in E-G, indicate the boundary between embryo and suspensor. Histological sections; apical end up.

ate from normal development. The period covered extends from the zygote (stage 1) to the mid-heart stage (stage 13) at which the primordia of the apical-basal pattern elements become recognisable (stages after Jürgens and Mayer, 1992).

In wild-type development, the zygote expands in the apical-basal axis before dividing asymmetrically to produce a smaller apical and a larger basal cell (Fig. 3A,B). The apical cell will give rise to most of the embryo while the basal cell will contribute the very basal end, including the quiescent center of the root meristem and the central portion of the root cap (Jürgens and Mayer, 1992). The apical cell is initially partitioned by a precise pattern of 'pseudo-cleavage' divisions resulting in the octant-stage embryo: two successive rounds of vertical divisions produce a quadrant of embryonic cells which then divide horizontally (Fig. 3C,D). The embryonic cells of the octant stage divide tangentially to give an outer layer of epidermal precursor cells and an inner cell mass (Fig. 3E). At this dermatogen stage, the uppermost derivative of the basal cell joins the embryonic cell group and becomes the hypophysis which divides asymmetrically (Fig. 3F), eventually giving rise to a characteristic arrangement of three layers each of 4 cells in the incipient root primordium of the mid-heart stage (Fig. 3G). The primordia of the seedling structures continue to grow until the embryo reaches maturity (Fig. 3H).

The earliest stage at which we observed abnormalities in *gnom* mutant embryos was the one-cell stage (stage 3 after Jürgens and Mayer, 1992). In contrast to wild-type where the apical cell is about one-third the size of the basal cell,

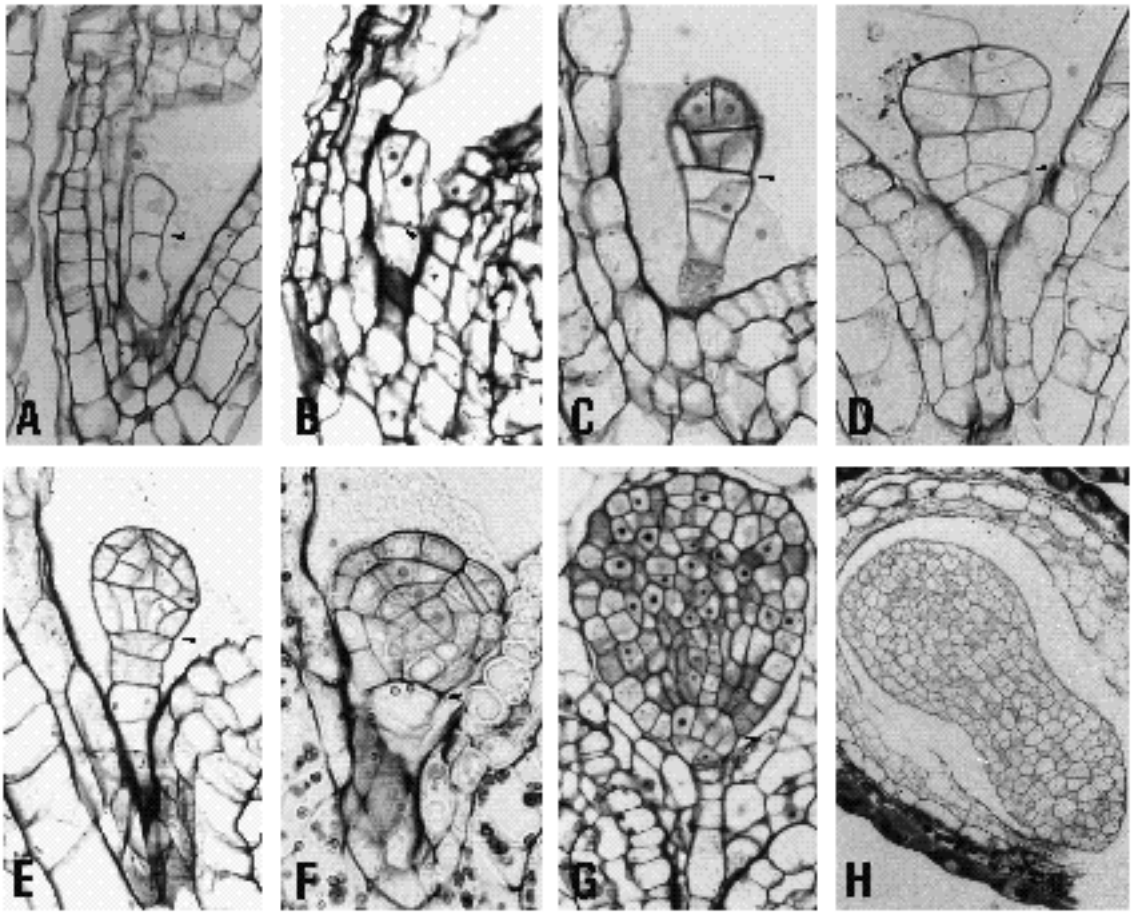

Fig. 4. *gnom* embryogenesis (staging is approximate). (A) One-cell stage: the plane of division is nearly symmetrical (compare with Fig. 3B). (B) Two-cell stage: the division of the apical cell is oblique (compare with Fig. 3C). (C) Abnormal four-cell stage; (D) enlarged octant stage; (E) dermatogen/early-globular stage; (F) mid/late-globular stage; (G) mid/late heart stage; (H) bent-cotyledon/mature-embryo stage. Small arrowheads in A-G mark the cell wall probably produced by the division of the zygote (see text). Histological sections; apical end up.

the apical cell was only slightly smaller than the basal cell and furthermore, the apical-basal dimension of the two cells was shorter than in wild-type (Fig. 4A). This observation suggests that the *gnom* gene is required before the asymmetric division of the zygote. Subsequently, the pattern of 'pseudo-cleavage' divisions is changed such that the apical cell may divide obliquely rather than vertically (Fig. 4B; other patterns of division will be described below). Early stages of mutant embryos were recognised by the absence of the outer layer of epidermal precursor cells while beyond the octant stage, the pattern of cell divisions is highly distorted, making it difficult to stage the mutant embryos accurately. Both the quadrant and the octant stage have more cells than the corresponding wild-type stages, and the patterns of cell arrangement are abnormal (Fig. 4C,D; see below). At the dermatogen or early-globular stage when the outer layer of epidermal precursor cells has formed, the junction between the basal end of the embryo and the suspensor looks abnormal (Fig. 4E). At this position, the uppermost derivative of the basal cell would normally become the hypophysis and divide asymmetrically, producing a lens-shaped cell and a larger basal daughter cell. These derivatives of the hypophysis were not observed in *gnom* mutant embryos at the mid/late-globular stage (compare Fig. 4F with 3F). Consistent with this basal defect, the mutant embryo lacks the characteristic cell arrangement that represents the incipient root primordium of the heart stage (compare Fig. 4G with 3G). In addition, there are no cotyledonary primordia bulging out at the apical end. Both the apical and the basal defect are reflected in the shape of the nearly mature embryo (Fig. 4H).

Mutant *gnom* seedlings show a variety of phenotypes, as described above. In order to determine the developmental basis for this phenotypic heterogeneity, we studied 'pseudo-cleavage' embryos of different *gnom* alleles (Fig. 5). These stages were developmentally equivalent to the two-cell or quadrant stages of wild-type embryos in which vertical cell walls occur within the embryonic cell group whereas a horizontal cell wall separates this group from the stalk-like arrangement of basal cell derivatives (similar to the embryo shown in Fig. 3C). In the mutants, however, there was no clear distinction between the embryonic group and the stalk, and the cells were arranged in a file or hook (Fig. 5). The cell wall separating the uppermost cell from the next was positioned abnormally and oriented horizontally (Fig. 5A,C) or obliquely (Fig. 5B,D,E) while the orientation was

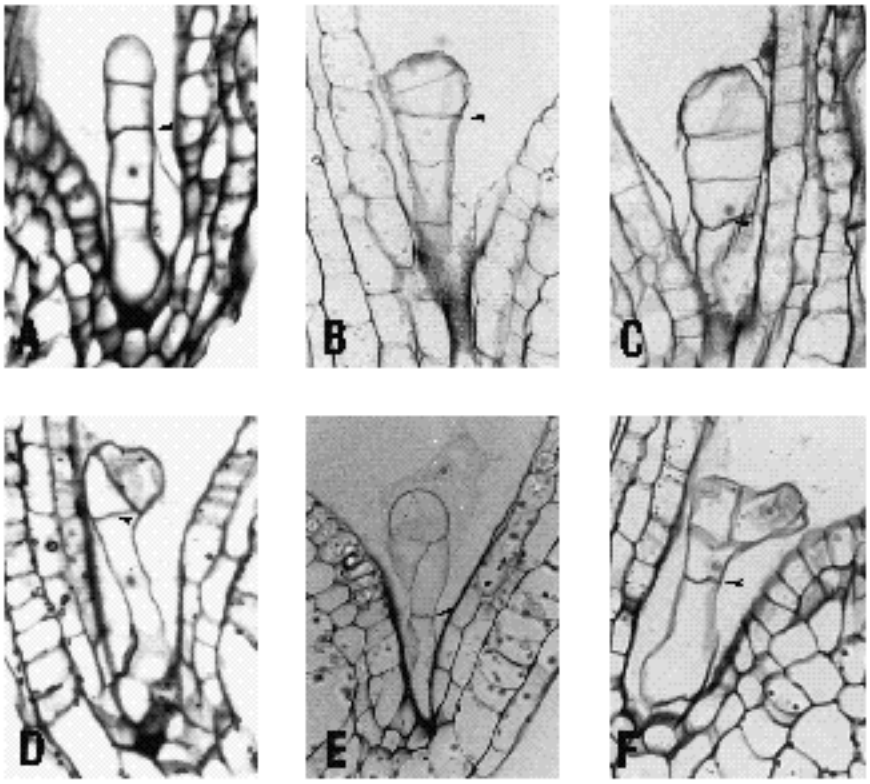

Fig. 5. Variable 'pseudo-cleavage' divisions in early *gnom* embryos from different alleles. (A-C,E) 4-13; (D) U75; (F) U263. Arrowheads mark the cell wall probably corresponding to the plane of division of the zygote (compare with Fig. 3C). Wild-type embryos from the same siliques were at about the same stages (two-cell or quadrant stages, corresponding to 5-8% of embryogenesis; see Jürgens and Mayer, 1992). Histological sections; apical end up.

vertical in the hook-shaped assemblage of cells (Fig. 5F). There was no preferential orientation in any one allele. As a control, other embryonic pattern mutants were analysed and such a deviation from the wild-type pattern of early cell division has not been observed, which suggests that this early phenotype is specific to the *gnom* gene (U. M., unpublished observations).

To determine if the abnormal 'pseudo-cleavage' divisions had developmental consequences, we analysed whole-mount preparations of slightly older mutant embryos that were probably equivalent to the dermatogen stage of wild-type (Fig. 6; compare with Fig. 3E). A variety of abnormal cellular patterns was observed, which corresponded well to the earlier heterogeneity. One of the types could be described as a superimposition of two octant/dermatogen stage embryos, with the upper half resembling the wild-type embryo more closely, while the lower half often showed abnormal cell patterns (Fig. 6A,B,D,E). The mid-line separating the two halves probably derived from the first 'pseudo-cleavage' division of the apical cell, which was often oriented perpendicular to the axis (Fig. 5A,C) or nearly so (Fig. 5B). Another type of mutant dermatogen stage had a continuous cell wall running obliquely across the embryo, which correlated with the oblique division of the apical cell during 'pseudo-cleavage' (compare Fig. 6C,F with 5D,E). These correlations suggest that the variable patterns of 'pseudo-cleavage' divisions have lasting effects on the development of *gnom* embryos and might well account for the phenotypic variability of mutant seedlings.

Tissue culture of *gnom* mutant seedlings

Most *gnom* mutant embryos do not form any root structures and all mutant seedlings lack a functional root meristem, as described above. We were interested to determine whether this defect is confined to embryogenesis or whether *gnom* mutants are also incapable of producing a root under experimental conditions. We first tested the response of intact mutant seedlings to culture on root-inducing medium (Fig. 7A-C). We observed limited disorganised growth but no root development in all phenotypes tested, including 'ball-shaped', 'cone-shaped' and 'weak' trans-heterozygous *gnom* seedlings that had reduced roots and root hairs before culture (Fig. 7C). Thus, intact mutant seedlings were incapable of promoting root development. We also noted a few abnormal leaves at the apical ends of those mutant seedlings that had clearly separate cotyledons but this effect was not due to culture on root-inducing medium because the seedlings behaved the same way when grown on soil.

We also studied the response of bisected seedlings to root-inducing tissue-culture conditions. It had previously been shown that late-stage somatic embryos of carrot, when bisected, can regenerate the missing root including the root meristem (Schiavone and Racusen, 1991). As a control, wild-type seedlings were bisected below the cotyledons and grown on root-inducing medium, and after 7-10 days culture the upper part had regenerated the missing root (Fig. 7D). When treated exactly the same way alongside their wild-type sibs, *gnom* seedlings responded differently: instead of forming the missing root, they produced green callus from the wound edge (Fig. 7E). The same result was observed for the 'weak' phenotype upon bisection and culture (data not shown). Mutant calli continued to grow for several months without producing roots (Fig. 7F). Thus, the *gnom* gene seems to be an essential component of processes leading to root formation, both in the embryo and in tissue culture.

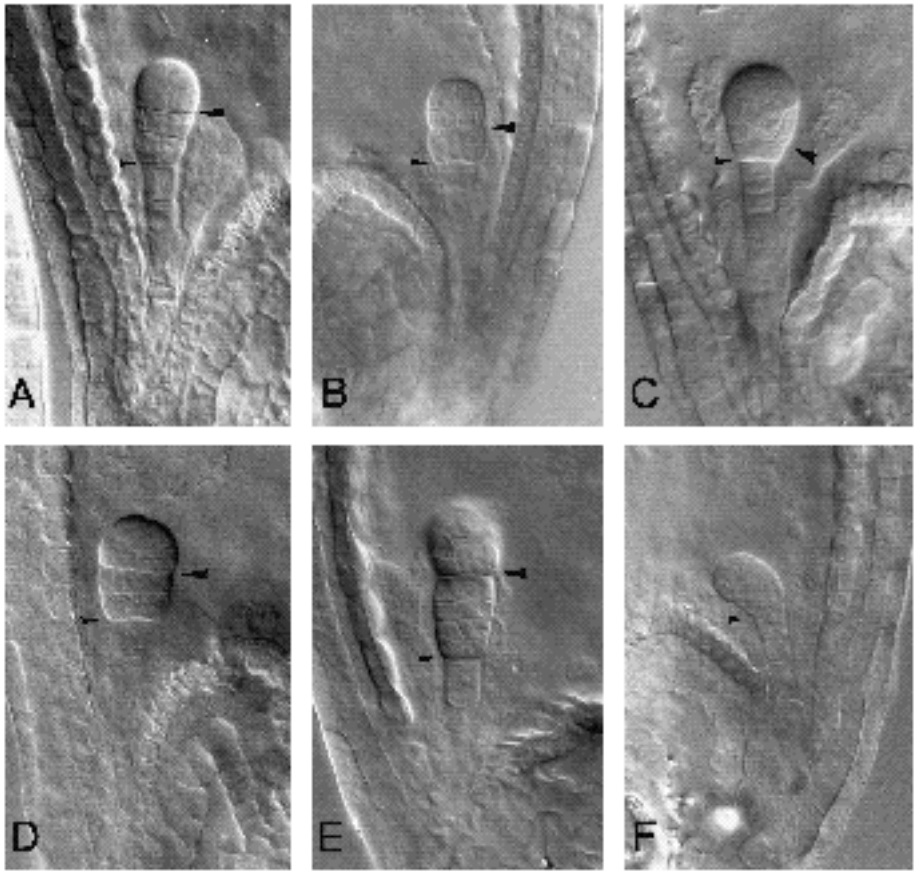

Fig. 6. Octant and dermatogen stages of *gnom* embryos from different alleles. (A) T339; (B) R310; (C) R310; (D) T339; (E) S28; (F) U87. Small arrowheads mark the cell walls probably corresponding to the plane of division of the zygote, large arrowheads (in A-E) the cell walls produced by the first 'pseudo-cleavage' division of the apical cell. Wild-type embryos from the same siliques were at about the same stages (octant or dermatogen stages, corresponding to 14-17% of embryogenesis; see Jürgens and Mayer, 1992). Whole-mount preparations, Nomarski optics; apical end up.

gnom is epistatic to *monopteros*

Apart from *gnom*, three other genes (*fackel*, *gurke*, *monopteros*) have been identified by mutant alleles that alter the apical-basal pattern of the embryo (Mayer et al., 1991). One way to reveal possible interactions between these genes is the analysis of double mutant phenotypes. When plants heterozygous for both *gnom* and *fackel* or *gurke* were selfed, no novel phenotypes were found among their seedling progeny (unpublished observation). The results are difficult to interpret because alleles of two unlinked genes had to be combined to give the double mutants. Whether one (and which) phenotype is epistatic to the other or the double mutant is lethal rests entirely on statistics: one-sixteenth of the progeny would be homozygous mutant for both genes as compared to three-sixteenth for each gene alone. By contrast, combining *gnom* (*gn*) with *monopteros* (*mp*) should give a clear result as these two genes are located in the same region of the first chromosome (this study; T. Berleth and G. J., unpublished data). Homozygous double mutants were expected to represent the majority of mutant seedling progeny if the parent plant was *gn mp* /+ + cis-heterozygous but to be very rare if the parent plant was *gn* +/+ *mp* trans-heterozygous, and this difference should make it very easy to discern any novel phenotype or epistasis.

The mutant phenotypes of *gn* and *mp* seedlings show partially overlapping pattern deletions. Both have been described as lacking the root whereas the hypocotyl is missing in *mp* but not in *gn* seedlings (Mayer et al., 1991; see Fig. 8). Among the seedling progeny of selfed trans-heterozygotes, *gn* and *mp* mutant phenotypes occurred in about the same proportions (Table 4a). However, mutant seedling progeny of selfed cis-heterozygotes showed at least 5× more often the *gn* than the *mp* phenotype, and no other mutant phenotype was observed (Table 4a). This result suggested that the *gn* phenotype is epistatic to the *mp* phenotype. In view of the implications for the interaction between the two genes, we checked the genotype of the putative double mutant in two ways. First, we grew normal-looking plants that had been produced by selfing and analysed their genotypes by progeny-testing. The result was consistent with the expected frequency distribution of genotypes as deduced from the genetic distance between the two genes (Table 4b). Second, crossing the putative double mutant *gn mp* / ++ with a *mp* / + heterozygote yielded nearly the expected proportion of *mp* mutant seedlings (compare Table 4c with 4a). Thus, the mutant phenotype of the *monopteros* gene cannot be recognised in *gnom monopteros* homozygous mutant seedlings.

DISCUSSION

Genetic dissection often provides a starting point for a mechanistic analysis of developmental processes. Relevant genes are recognised by specific mutant phenotypes but in order to assign each gene a particular role in the process, the relationship between changes in gene activity and resul-

Table 4. Analysis of *gnom monopteros* double mutants

(a) *Progeny of selfed* gn + / + mp *plant*

Genotype of F_1 plant	Number observed	Number expected*	Number of F_2 seedlings	% seedling phenotypes gn	mp	+
gn + / + mp	103	104.0	6989	25.5	22.3	52.2
gn + / + +	12	11.5	735	23.4	—	76.6
+ + / + mp	12	11.5	742	—	19.8	80.2
gn mp / + +	1	1.3	85	20.0	3.5	76.5
+ + / + +	1	0.7	(>50)	—	—	100
∑	129	129.0				

*based on genetic distance of 10 cM between *gnom* and *monopteros*.

(b) *Adult progeny of selfed* gn mp /++

Genotype	Number observed	Number expected*
gn mp / + +	16	20
+ + / + +	13	10
gn + / + +	7	5
+ *mp* / + +	3	5
gn + / + *mp*	0	<1
other	1†	—
∑	40	40

*based on genetic distance of 10 cM between *gnom* and *monopteros*.
†probably *gn mp* / ++ / + *mp*.

(c) *Testcross of* gn mp / + +

Parental genotypes	Number of F_1 seedlings	% seedling phenotypes gn	mp	+
gn mp / + + selfed*	121	15.7	1.7	82.6
gn mp / + + × + *mp* / + +	79	—	20.2	79.8

*different flowers of the same plant used in the testcross (control).

tant mutant phenotypes has to be clarified. The aim of the present study was to explore the role that the *gnom* gene plays in apical-basal pattern formation in the *Arabidopsis* embryo. We will discuss our results from three different angles: (1) the lack-of-function phenotype, (2) the developmental basis for the phenotypic heterogeneity of mutant seedlings and (3) the primary effect of *gnom* gene activity at the cellular level.

Complete inactivation of the *gnom* gene results in phenotypic heterogeneity

The probable wild-type function of a gene can reliably be inferred from the phenotypes of mutant alleles if the true null phenotype is known and if the mutant alleles can be arranged in a phenotypic series of increasing deviation from the wild-type pattern, reflecting different amounts of residual gene activity. In *Arabidopsis*, this has been established for the *leafy* gene which controls the identity of the floral meristem (Weigel et al., 1992). Of 7 EMS-induced alleles analysed at the molecular level, 3 showed a strong phenotype and were associated with nonsense mutations generating a mutant peptide less than 10% the size of the wild-type protein, whereas intermediate and weak phenotypes were due to different missense mutations. The molecular analysis of the *chl3* gene, which codes for nitrate reductase (NR) in *Arabidopsis*, tells a different story: 3 strong EMS-induced alleles do produce full-length protein and yet have the same low level of enzyme activity as physical deletions of the *chl3* gene (Wilkinson and Crawford, 1991). In higher plants, NR appears to be a homodimeric protein with three distinct domains, and intragenic complementation has been observed between subgroups of NR-deficient *nia* mutants in *Nicotiana plumbaginifolia*: complementation occurs only between alleles affecting different domains of the protein, both in vivo and in vitro (Caboche and Rouzé, 1990). We interpret the *gnom* complementation data along these lines.

Partial complementation between group A and group B alleles, as indicated by the weak phenotype of their hybrids, may be taken as evidence for the association of identical subunits to give active *gnom* gene product in the wild-type. Group A and B alleles may thus produce non-functional but full-length proteins due to amino acid changes whereas the non-complementing group C alleles may produce truncated proteins due to stop codons. This interpretation is consistent with the known mutagenic effects of EMS, as discussed above. That group A alleles are more numerous than group B alleles might reflect different target sizes for EMS mutagenesis. Whether these targets also correspond to distinct domains of the gene product has to await molecular characterisation of the *gnom* gene.

In our interpretation, group C alleles are true null mutations although they produce essentially the same phenotypic range of mutant seedlings as the other alleles. For all alleles, 'ball-shaped' seedlings which do not express apical-basal polarity represent a minority, and one of the B group alleles, U87, even produced a larger proportion of 'ball-shaped' seedlings than the group C alleles (see Table 3).

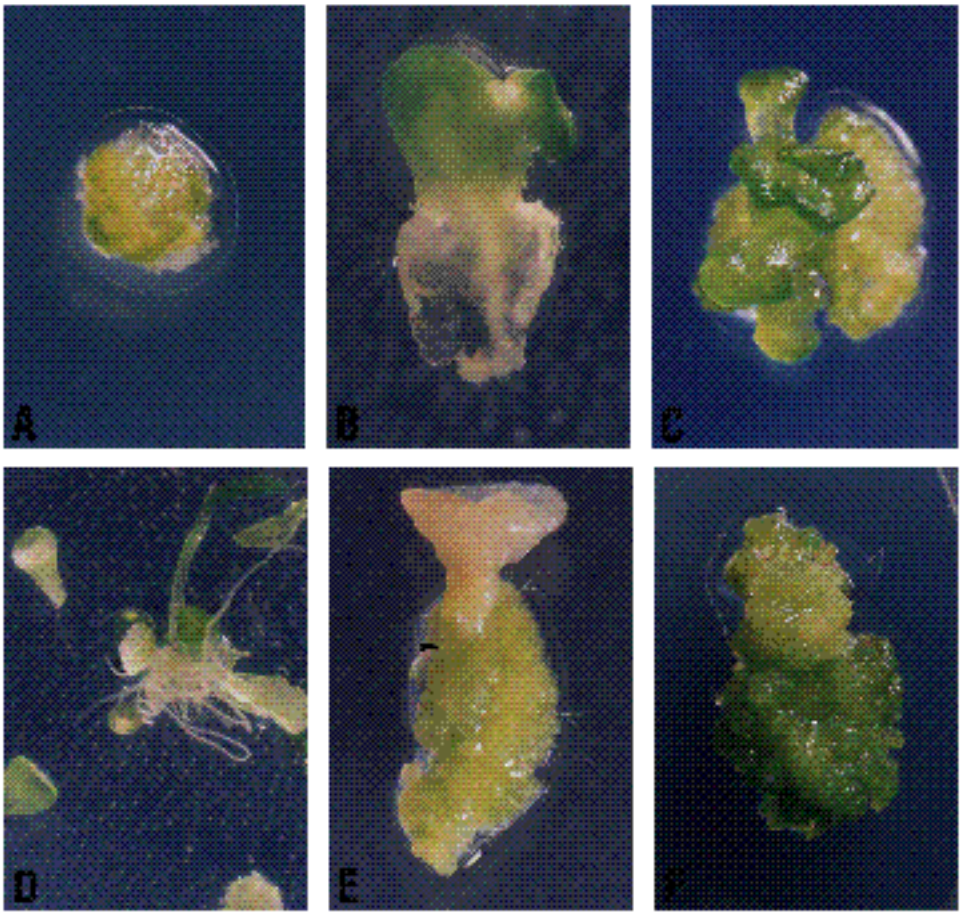

Fig. 7. Response of seedlings to culture in root-inducing medium. (A-C) intact *gnom* seedlings: (A) 'ball-shaped' (compare Fig. 1G); (B) 'cone-shaped' (compare Fig. 1B); (C) 'weak' phenotype (compare Fig. 1K). (D,E) bisected seedlings: (D) wild-type: regeneration of root by apical part after bisection below the cotyledons; (E) 'cone-shaped' *gnom* seedling (compare Fig. 1D): formation of callus by apical part after bisection at the level indicated (arrowhead); (F) callus derived from (E) after several months of culture.

Any phenotypic differences between the 24 alleles should thus not reflect differences in residual *gnom* gene activity. These considerations suggest to us that the *gnom* gene is not absolutely required for generating the apical-basal polarity in the embryo. Rather, complete inactivation of the *gnom* gene results in a range of seedling phenotypes, differentiating variable portions of the apical-basal pattern: while the root end of the pattern is always deleted, the apical end seems to be less sensitive.

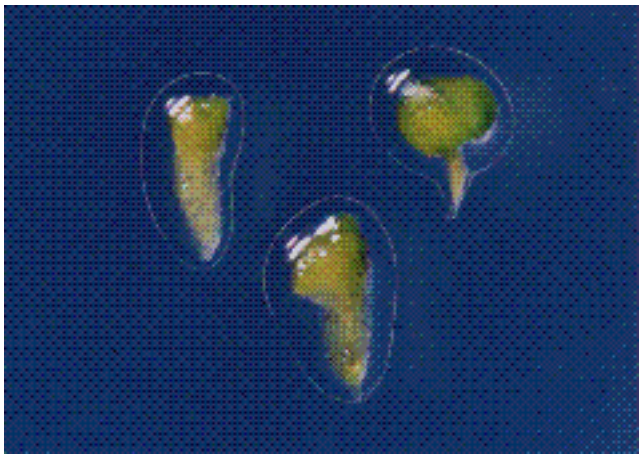

Fig. 8. Mutant seedling phenotypes produced by selfing of *gn mp / + + cis*-heterozygous plant. *gnom* is shown on the left, *monopteros* on the right; the seedling in the centre may be doubly mutant but cannot phenotypically be distinguished from *gnom*.

Developmental basis for the phenotypic heterogeneity of *gnom* seedlings

The origin of mutant seedling phenotypes cannot be determined by live recording of developing embryos as plant embryos develop inside the ovule which is enclosed in a fruit. However, the development of abnormal embryos can be reconstructed from the analysis of successively younger stages of embryogenesis, and in this way the origin of the seedling phenotype is traced back to the earliest stage at which deviations from normal can be recognised. Although this procedure relies on topographical correlations, the lack of cell movements in plant embryogenesis facilitates the analysis, especially as the regularity of normal embryogenesis in *Arabidopsis* and related crucifers has been well documented (*Arabidopsis*: Vandendries, 1909; Müller, 1963; Meinke and Sussex, 1979; Mansfield and Briarty, 1991; Jürgens and Mayer, 1992; *Capsella*: Schulz and Jensen, 1968; *Brassica napus*: Tykarska, 1976, 1979). Early embryogenesis, in particular, is characterised by invariant cell division patterns from the asymmetric division of the zygote to the 'pseudo-cleavage' division cycles of the apical daughter cell to the tangential divisions of the octant stage and by the precise division pattern of the hypophysis generating the incipient root primordium of the heart stage.

We observed abnormal patterns of 'pseudo-cleavage'

divisions in early *gnom* mutant embryos which could be correlated with 'octant stages' consisting of about twice the normal number of cells (Figs 5, 6). The deviations from the normal pattern were variable although a general tendency was clear: the first 'pseudo-cleavage' division of the apical cell, which is normally oriented vertically, tended to form perpendicular to the apical-basal axis. This change was consistently noticed for each allele analysed and may thus reflect the lack of *gnom* gene activity in the early embryo. The plane of plant cell division is thought to be determined by the dimensions of the cell such that the longest axis is partitioned (Lyndon, 1990; Lloyd, 1991). It may thus simply be the relative dimensions of the apical cell that generate the regular pattern of the three rounds of 'pseudo-cleavage' divisions (Mansfield and Briarty, 1991). Our data on one-cell stages of *gnom* embryos are consistent with this view: the apical cell was more expanded in the apical-basal axis at the expense of the basal cell, with the former approaching the size of the latter (Fig. 4). It thus appears that the division of the zygote itself, which is asymmetric in the wild-type, tends to become symmetric in *gnom* mutant embryos. The zygote normally expands about three-fold in the apical-basal axis, moving the nucleus to the level of the future division plane (Webb and Gunning, 1991; Mansfield and Briarty, 1991). Occasionally, we observed potentially mutant zygotes that enlarged but did not expand much in the axis and the nucleus was nearly in the centre of the cell (unpublished observations). If this observation could be substantiated, the *gnom* gene would be required in the zygote for proper cell elongation and positioning of the nucleus.

In summary, the *gnom* gene seems to act before the one-cell stage. In the absence of *gnom* activity, the division of the zygote generates an abnormally large apical cell and, depending on the cell size, the plane of the first 'pseudo-cleavage' division may change in different ways, which seems to be the developmental basis for the phenotypic heterogeneity of mutant seedlings.

Primary effect of *gnom* gene activity at the cellular level

Our developmental analysis of *gnom* mutant embryos has provided evidence for the following deviations from wild-type embryogenesis. (1) The plane of division of the zygote is altered, which entails an abnormal 'pseudo-cleavage' pattern of its apical daughter cell. (2) Root formation, which normally proceeds via the hypophysis and its characteristic assemblage of derivatives, is not initiated. (3) Variable apical defects become apparent in the heart-shaped embryo when the cotyledonary primordia fail to form or do not form properly. To explain these diverse effects, we propose that *gnom* gene activity specifically promotes the asymmetric division of the zygote and that the 'default' state of (nearly) symmetrical division entails the other embryonic defects. The division of the zygote is special in several respects, as will be discussed below, and attributing the primary defect to the failure of proper division of the zygote is thus the simplest assumption. We cannot, however, rule out the possibility that the *gnom* gene is required again later in the embryo to promote other asymmetric divisions. This effect would have to be specific because, for example, the asymmetric divisions generating the guard cells of the stomata are not affected in *gnom* mutants. By contrast, the hypophysis, which normally generates two unequal daughter cells, does not form in *gnom* mutant embryos and it is thus not clear whether *gnom* activity also promotes the asymmetric division of the hypophysis. This difficulty is in part related to the fact that the hypophysis is recognised by its pattern of cell division. If this pattern is abnormal the cell in its place may not be the hypophysis, due to an earlier defect, or the hypophysis may be present but non-functional, due to some intrinsic defect. The latter possibility would account for the weak *gnom* phenotype if the hypophysis is required for organising root development, which has not been demonstrated.

In addition to the alterations in embryogenesis, we made the unexpected observation that bisected *gnom* mutant seedlings were unable to produce a root from the wound edge, forming callus instead.

More extensive tissue-culture studies were done on the *emb30* mutant, which we have shown to be a *gnom* allele, and essentially the same failure of root formation was observed although different plant material was cultured on various media (Baus et al., 1986). To account for this effect within the frame-work of our hypothesis, we have to presume that the change from disorganised to organised growth may initially require a *gnom* dependent change from symmetric to asymmetric cell division. Although the cellular changes associated with the regeneration of root from bisected seedlings have not been analysed in detail (Schiavone and Racusen, 1991), other experiments are consistent with our presumption. In maize, for example, removal of the root cap and the quiescent centre, which in *Arabidopsis* are derived from the hypophysis of the embryo, can be compensated by the root stump which is able to regenerate a complete root apex with a new quiescent centre (Feldman, 1976), and an excised quiescent centre can regenerate a complete root apex (Feldman and Torrey, 1976). If the cellular events leading to the formation of the quiescent centre are the same in embryogenesis and root regeneration, the *gnom* gene might be involved in both, which would argue for repeated *gnom* gene activity in the developing embryo.

In summary, the primary effect of *gnom* gene activity at the cellular level might be to promote asymmetric division of specific cells, including the zygote and possibly the hypophysis in normal embryogenesis and as yet unidentified cells involved in regenerating the root.

Developmental significance of the asymmetric division of the zygote

The zygote divides perpendicular to the future apical-basal axis to give two cells of different sizes and developmental fates, a common feature to most flowering plants and the brown alga, *Fucus* (Johri, 1984; Quatrano, 1978). A differentiative division of the zygote also occurs in the nematode, *Caenorhabditis elegans*, to give a large anterior cell and a small posterior cell associated with different developmental fates (review by Schnabel, 1991). In all these cases, the cytoskeleton undergoes spatial reorganisation before the zygote enters mitosis (Webb and Gunning, 1991; Kropf et al., 1989; Quatrano, 1990; Hill and Strome, 1990).

It has been shown in *Caenorhabditis* that perturbation of the cytoskeleton affects both the division plane of the zygote and the developmental fates of the daughter cells (Hill and Strome, 1990). In *Fucus*, cell polarity has been manipulated by external gradients which can lead to a bipolar zygote with two rhizoid ends (review by Quatrano, 1978). Although experimental manipulation of flowering plant zygotes has not been done for technical reasons, the 'double root' phenotype, which was observed in *Arabidopsis* after EMS mutagenesis (Jürgens et al., 1991), suggests that 'determinants' are also distributed in the plant zygote and probably fixed by the asymmetric division. Since in *Arabidopsis* the cortical microtubules become aligned perpendicular to the direction of cell expansion as the zygote begins to elongate (Webb and Gunning, 1991) and this reorganisation may be altered in *gnom* mutants, it is tempting to speculate that the *gnom* gene is also involved in the distribution of 'determinants'. The phenotype of the *gnom monopteros* double mutant points in this direction: if the *monopteros* gene is required for making hypocotyl and if the double mutant is indistinguishable from the *gnom* mutant, the *gnom* mutant does not differentiate hypocotyl. Indeed, the hypocotyl lacks any specific cell feature and is usually defined as the axis between the cotyledons and the root. It might thus well be that *gnom* mutants differentiate a 'ground state' rather than a hypocotyl, suggesting that the *gnom* gene not only promotes the asymmetric division of the zygote but is also required for the generation of developmental fates.

We are especially grateful to Thomas Berleth for demonstrating that roots can be regenerated from wounded wild-type seedlings, we also thank David W. Meinke for sending us seeds from the *emb30* line, Maarten Koornneef for seeds from various marker lines, and Thomas Berleth, Max Busch, Hans-Georg Frohnhöfer, Martin Hülskamp, Thomas Laux, Wolfgang Lukowitz and Simon Miséra for valuable comments on the manuscript. This work was supported by the Deutsche Forschungsgemeinschaft.

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(Accepted 18 September 1992)