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Embryogenic Transformation of the Suspensor in *twin*, a Polyembryonic Mutant of *Arabidopsis*

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Spontaneous twinning is a widespread but infrequent phenomenon in higher plants. We describe here a mutant of *Arabidopsis thaliana, twin,* that yields an unusually high frequency of viable twin and occasional triplet seedlings. Supernumerary embryos of *twin* arise through a novel mechanism: transformation of cells within the suspensor, a differentiated structure established early in embryogenesis. Twin embryos develop in tandem within the seed, connected by intact segments of the suspensor. Transformed suspensor cells appear to duplicate the patterns of cell division and developmental pathways characteristic of zygotic embryogenesis. In addition to polyembryony, mutant embryos exhibit a number of developmental defects, including irregular patterns of cell division and abnormal morphology. The *TWIN* locus therefore appears to be required for normal development of the embryo proper as well as suppression of embryogenic potential in the suspensor. The development of viable secondary embryos in *twin* demonstrates that cells of the *Arabidopsis* suspensor can successfully establish embryonic polarity and complete the full spectrum of developmental programs normally restricted to the embryo proper. In addition, the *twin* phenotype indicates that disruption of a single genetic locus can result in the conversion of a single terminally differentiated cell type to an embryogenic state.

INTRODUCTION

Embryogenesis in higher plants is a complex process dependent on coordination of specific genetic programs and proper communication between different parts of the developing seed. The analysis of mutants impaired in morphogenesis and cell differentiation provides a powerful strategy for understanding the genetic mechanisms underlying plant embryo development (Meinke, 1991a). A large number of embryonic mutants have been identified in maize and *Arabidopsis* (Clark and Sheridan, 1991; Meinke, 1991b). Mutants defective in specific aspects of embryo development, such as pattern formation (Mayer *et al.*, 1991, 1993; Berleth and Jürgens, 1993), meristem establishment (Barton and Poethig, 1993), and maturation programs (Meinke, 1992; Meinke *et al.*, 1994; Keith *et al.*, 1994), have recently been characterized in detail.

Twinning represents a fascinating and unusual example of embryogenesis gone awry. Polyembryony, although a rare event in higher plants, occurs in a broad range of taxa (Tisserat *et al.*, 1979). Supernumerary embryos can arise spontaneously from a number of sources within the seed, including maternal, gametophytic, and zygotically derived tissues (Tisserat *et al.*, 1979; Johri *et al.*, 1992). In most cases, supernumerary embryos undergo limited development and are inviable. Twinning has also been induced in some species by exposing developing seeds to γ-radiation (Akhundova *et al.*, 1979) or treatment with growth regulators (Haccius, 1955). The origin of twin embryos in such cases has not been determined.

We describe here an embryo-defective mutant of *Arabidopsis*, *twin* (*twn*), that produces a high percentage of twin seedlings. *Twn* is a pleiotropic mutant that displays incomplete penetrance and a wide range of defects in embryonic cell division, morphological development, and seedling growth. The most striking phenotype, however, is polyembryony. Microscopy of developing seeds has revealed that twins arise during embryogenesis by a unique mechanism. Cells of the suspensor undergo embryogenic transformation, resulting in formation of a secondary embryo capable of surviving seed desiccation and germinating to produce a viable seedling. *Twn* demonstrates that differentiated cells of the suspensor maintain the capacity to duplicate the pathway of zygotic embryogenesis. We propose that the *twn* mutation relieves an inhibitory effect normally imposed on the suspensor by the embryo proper, thereby allowing the suspensor to express its full developmental potential. The wild-type *TWN* gene therefore appears to be essential for normal development of the embryo proper, suppression of embryogenic potential in the suspensor, and maintenance of suspensor cell identity during *Arabidopsis* development.

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MATERIALS AND METHODS

Plant Material

Heterozygous plants (ecotype Wassilewskija) were grown in soil as described by Errampalli *et al.* (1991). For germination studies, mutant seeds were removed from mature siliques of self-pollinated *twn* heterozygotes and homozygotes, surface sterilized, and plated on germination media containing the inorganic salts of Murashige and Skoog (1962), 3% glucose, and 0.8% agar as described by Baus *et al.* (1986). Plates were refrigerated for 4 days and germinated at 22°C under 16 hr light/8 hr dark cycles. Homozygous mutant lines were maintained by transplanting seedlings to soil 2 to 3 weeks after plating.

Genetic Analysis

To determine the chromosomal location of *twn*, homozygotes were crossed into established mapping lines (DP23, DP24, and DP28/*hy*2), each of which has recessive visible markers located on different chromosomes (Patton *et al.*, 1991; Castle *et al.*, 1993). F_1 plants were selfed and F_2 seeds homozygous for *twn* identified by their abnormal seed phenotype. These mutant seeds were then germinated, grown to maturity, and scored for segregation of visible markers. Recessive markers unlinked to the *twn* locus were expected to be visible in 25% of the F_2 *twn/twn* population. A significantly lower percentage of any marker phenotype indicated linkage to *twn*. Linkage data were analyzed using the EF method described by Patton *et al.* (1991).

Penetrance of the *twn* mutation was determined by scoring for the presence of abnormal seeds in siliques of self-pollinated *TWN/twn* and *twn/twn* plants. Seeds were examined with a dissecting microscope or with a compound microscope equipped with Nomarski optics. Seeds that appeared shrunken, off-colored, or generally distorted under a dissecting microscope were scored as embryo-defective. Nomarski optics allowed scoring of progeny seeds by direct visualization of embryo phenotypes. In experiments addressing incomplete penetrance of the embryo-defective phenotype, embryo phenotypes in seeds produced by phenotypically normal and abnormal *twn/twn* plants were compared.

Twinning frequencies were determined by Nomarski optics or by germination of mutant seeds collected from *TWN/twn* and *twn/twn* plants. Over 2000 seeds obtained from 16 mutant subfamilies were subjected to Nomarski analysis. Outcrosses to Landsberg *erecta* and wild-type Wassilewskija (WS) ecotypes were carried out with homozygous mutant plants. In WS outcrosses, *twn/twn* plants that had germinated both as single seedlings and twins were used. F_2 progeny from 5 to 20 F_1 plants from each cross were characterized by Nomarski optics or with germination studies to determine penetrance, twinning percentage, and range of phenotypes.

Microscopy

Intact seeds were removed from immature siliques, fixed for 15 to 30 min in Histochoice fixative (Amresco, Solon, OH), transferred to a microscope slide, and cleared in Hoyer's solution (7.5 g gum arabic, 5 ml glycerin, 100 g chloral hydrate, and 30 ml H_2O). Seeds at early stages of development were cleared for several hours; seeds containing cotyledon stage embryos were cleared overnight or for several days. Cleared seeds were examined with an Olympus BHS compound microscope equipped with Nomarski interference optics.

RESULTS

Mutant Isolation

Twn was originally identified by screening for abnormal seeds in siliques of transgenic *Arabidopsis* lines produced by *Agrobacterium*-mediated seed transformation (Feldmann, 1991; Castle *et al.*, 1993). Heterozygous plants resembled wild type except for the production of abnormal seeds following self-pollination. Seeds containing homozygous mutant embryos were often small, discolored, or uneven in shape. Heterozygotes were nopaline negative and sensitive to kanamycin, indicating that a functional T-DNA insert was not associated with the mutation (Errampalli *et al.*, 1991; Castle *et al.*, 1993). This result is consistent with the finding that only 35% of the embryo-defective mutants identified in this population of mutagenized plants are tagged with a T-DNA insert (Castle *et al.*, 1993).

To investigate the germination potential of mutant embryos, we plated homozygous *twn* seeds on basal germination media. The resulting seedlings displayed a variety of developmental defects. Many mutant plants were small, distorted, or slow to green (Fig. 1). Others appeared phenotypically normal, suggesting that penetrance of the *twn* mutation is incomplete. Within the population of abnormal seedlings, developmental defects were observed in different combinations and with varying degrees of severity, resulting in a broad continuum of mutant seedling phenotypes. Most seedlings were viable, allowing the establishment of homozygous mutant lines. The most striking phenotype was the germination of twins and occasional triplets (Fig. 1). Approximately 9% of mutant seeds gave rise to multiple seedlings (Table 1). Spontaneous twinning in wild-type *Arabidopsis* occurs at a frequency of 0.02% (Akhundova *et al.*, 1979). The frequency of twinning in this mutant is therefore 400 to 500 times that of wild type. We have not previously observed germination of twins among the 250 embryo-defective mutants characterized in our laboratory (Meinke, 1994).

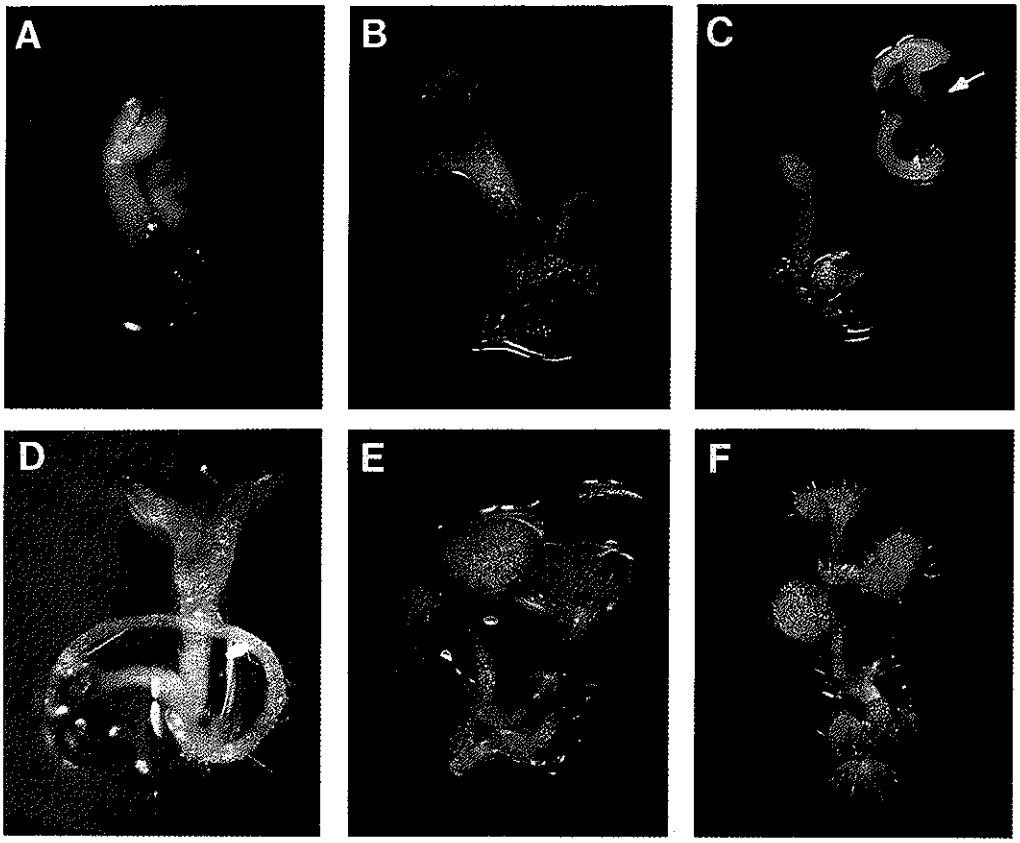

FIG. 1. Germination of mutant seedlings from dry seed. (A,B) Two sets of twins with abnormal pigmentation and irregular cotyledons. (C) Triplets. The largest seedling has reduced cotyledons; the two smaller siblings are attached at their root tips (arrow). (D–F) Viable twin pairs: (D) Siblings differing in size, the smaller with very reduced cotyledons. (E) Seedling on left resembles wild type; its sibling has one large cotyledon. (F) Twin plants that resemble wild type.

Genetic Analysis

Several lines of genetic evidence indicate that the polyembryony and other developmental abnormalities associated with *twn* result from a single recessive mutation with incomplete penetrance and pleiotropic effects. Mapping with visible markers localized the *twn* mutation to a single locus (Table 2). *Twn* mapped to the lower arm of chromosome 5, linked to the *tt3* and *yi* loci. Segregation analysis of seeds from self-pollinated heterozygotes and homozygotes indicated that *twn* behaves as

TABLE 1
FREQUENCY OF POLYEMBRYONY IN MUTANT SEEDS

Seed populations examined	Twins observed	Total seeds examined	Percent polyembryony
twn/twn seed collected from original mutant lines[a]	95	1052	9.0
Total F_2 seed from *twn/twn* × WS outcross[b,c]	10	454	2.2
Total F_2 seed from *twn/twn* × WS outcross[b,d]	13	590	2.2
Total F_2 seed from *twn/twn* × Ler outcross[b]	13	845	1.5

[a] *twn/twn* seeds were collected from *TWN/twn* and *twn/twn* plants following self-pollination. Twinning percentage was determined by germination studies.

[b] Homozygous mutant plants were crossed into Landsberg *erecta* (Ler) and Wassilewskija (WS) ecotypes. F_1 heterozygotes were selfed and F_2 seed populations scored for polyembryony by Nomarski optics. Approximately 25% of these F_2 seeds were homozygous for the *twn* mutation. The frequency of twinning was therefore expected to be 25% of that observed in homozygous mutant lines.

[c] Outcrosses carried out with *twn/twn* parent plants that had germinated as twins.

[d] Outcrosses carried out with *twn/twn* parent plants that had germinated as single seedlings.

TABLE 2
LINKAGE ANALYSIS OF THE *TWN* LOCUS

Marker (chromosome)	F_2 *twn* plants screened	Plants with marker phenotype	Expected if unlinked to *twn*	χ^2
*dis*1 (1)	113	24	28	0.85
*ch*1 (1)	95	24	24	0.00
*clv*2 (1)	94	25	24	0.13
er (2)	263	73	66	1.07
*gl*1 (3)	95	30	24	2.19
*hy*2 (3)	113	28	28	0.00
*tt*5 (3)	90	28	23	1.79
*cer*2 (4)	95	25	24	0.09
bp (4)	70	17	18	0.02
ttg (5)	89	18	22	1.08
*tt*3 (5)	90	7	23	14.24*
yi (5)	89	6	22	15.82*

Note. Homozygous *twn* plants were crossed into mapping lines carrying recessive visible markers located on each chromosome. Segregation of marker phenotypes was then observed in *twn/twn* F_2 progeny. Unlinked markers were predicted to be visible in 25% of the F_2 *twn/twn* population. χ^2 represents deviation from this predicted segregation behavior. Two markers (*) deviated significantly from the predicted segregation pattern ($P \leq 0.005$), indicating linkage to *twn*.

a recessive mutation with approximately 50% penetrance (Table 3). To confirm that the low ratios of phenotypically abnormal seeds in *twn* are due to incomplete penetrance of the embryo-defective phenotype, progeny of phenotypically normal and abnormal *twn/twn* plants were compared (Table 3). Regardless of the phenotype of the parent plant, approximately 50% of the progeny appeared abnormal, displaying the full continuum of mutant phenotypes observed in germination studies. Segregation behavior of *twn* remained unchanged after outcrossing into Landsberg *erecta* and wild-type Wassilewskija backgrounds (Table 3). Thus, the low ratio of embryo-defectives in *twn* heterozygotes and homozygotes is due to incomplete penetrance of the *twn* mutation. Nomarski analysis of over 700 mutant seeds from subsequent generations yielded similar results, confirming that ~50% penetrance is a stable characteristic of the *twn* defect.

The polyembryonic phenotype of *twn* was fully heritable and was passed on by mutant plants that had germinated both as twins and as single seedlings. Extensive germination studies, as well as Nomarski analysis of over 2000 mutant seeds, indicated that twins and *twn/twn* single plants consistently gave rise to comparable numbers of polyembryonic seeds following self-pollination. Outcrosses to Landsberg *erecta* and Wassilewskija ecotypes confirmed the stability of the polyembryonic phenotype and the frequency of twinning (Table 1).

Developmental Analysis

To determine the origin of twin seedlings, we compared embryo development in wild-type (Fig. 2) and mutant seeds (Fig. 3). Most *twn* embryos appeared normal through the early globular stages of development. Proper asymmetric division of the zygote gave rise to a two-celled proembryo consisting of an elongate basal cell and a round terminal cell (Fig. 3A). At the preglobular stage, mutant seeds contained a single embryo proper accompanied by a developing suspensor. No supernumerary embryos were observed in approximately 400 preglobular seeds examined by Nomarski optics. Thus, twins were not arising from multiple fertilization events or by splitting of the zygote.

Examination of older *twn* seeds revealed the origin of supernumerary embryos. Twin embryos arose by transformation within the suspensor (Figs. 3B–3F). The suspensor is the first differentiated organ to form during plant development (see Fig. 2B). In *Arabidopsis* it consists of a single column of five to eight cells that supplies the developing embryo with nutrients and possibly growth regulators (reviewed by Yeung and Meinke, 1993). Normally, as embryos reach more advanced stages of development, suspensor cells undergo programmed cell death and the suspensor disintegrates (Fig. 2D). In *twn*, secondary embryos developed when cell proliferation within the suspensor gave rise to multicellular structures with unmistakable embryonic features (Figs. 3B–3D). Sibling embryos (twins as well

TABLE 3
INCOMPLETE PENETRANCE OF THE EMBRYO-DEFECTIVE PHENOTYPE IN MUTANT SEEDS

Parental genotype	Nature of parental plants	Phenotype of progeny seeds[a] Abnormal	Total	% Abnormal
twn/TWN	Original mutant lines (WS)	153	1085	14
twn/TWN	F_1 from outcrosses to WS[b]	309	1849	17
twn/TWN	F_1 from outcrosses to Ler[b]	138	845	16
twn/twn	Original mutant lines (WS)	468	1052	44
twn/twn	Phenotypically abnormal[c]	77	143	54
twn/twn	Phenotypically normal[c]	126	276	46

[a] Abnormal seeds identified following self-pollination of *twn/TWN* and *twn/twn* plants. A recessive embryo-defective mutation with complete penetrance should result in 25% abnormal seeds in siliques of selfed heterozygotes or 100% abnormal seeds in siliques of selfed homozygotes.

[b] Homozygous mutant plants were crossed into Landsberg *erecta* (Ler) and Wassilewskija (WS) ecotypes. Total F_2 seeds produced following self-pollination of resulting F_1 heterozygotes were scored.

[c] Progeny of phenotypically normal *twn/twn* plants were compared to progeny of plants that had appeared abnormal as seedlings.

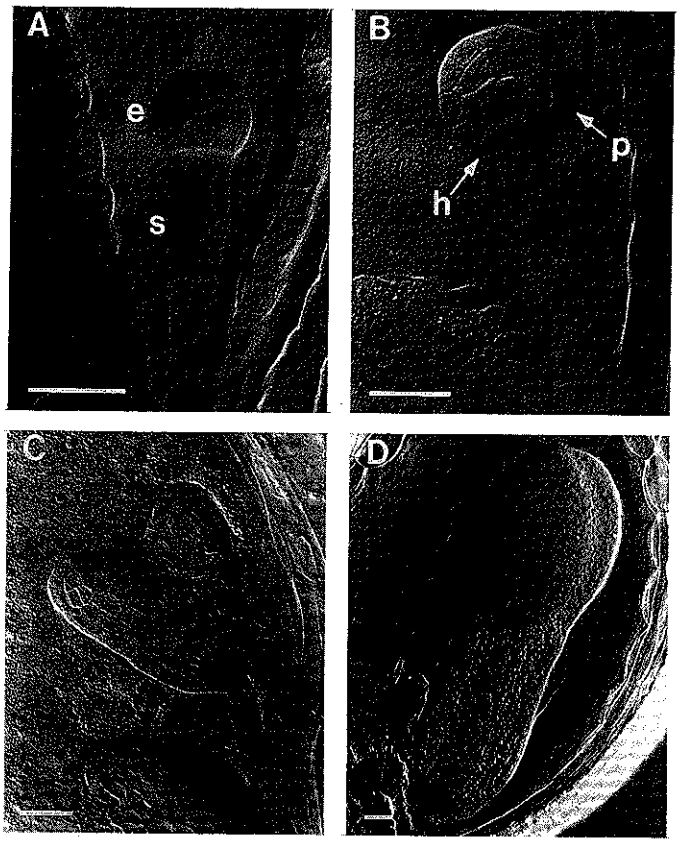

FIG. 2. Wild-type *Arabidopsis* embryo development. Seeds were harvested at various stages of embryo development and viewed with Nomarski optics as described under Materials and Methods. (A) Four-celled preglobular embryo proper (e) accompanied by a developing suspensor (s). Characteristic longitudinal divisions (Mansfield and Briarty, 1991) define cells of the embryo proper (only two cells are visible in focal plane). (B) Globular stage embryo. The suspensor is already fully formed; cells of the protoderm (p) are evident in the embryo proper. A distinct arc at the top of the hypophyseal cell (h) marks the division between the embryo proper and suspensor. (C) Heart stage embryo. Cotyledon primordia give the embryo bilateral symmetry. Specialized cell types, including the protoderm and provascular tissues, are now present in the embryo proper. The root meristem, derived from the suspensor, is developing from the hypophysis. (D) Linear stage embryo. The embryo, now autotrophic, has begun to enlarge to fill the seed. Cells of the suspensor have undergone programmed cell death and are no longer visible. Scale bar, 20 μm.

as triplets) were observed aligned in tandem, connected by segments of the suspensor. As development progressed, suspensor connections between embryos degenerated (Fig. 3F). Occasionally, young seedlings remained connected through germination (Fig. 1C). Transformation could originate at any suspensor cell, as illustrated by the variable location of secondary embryos within the suspensor and by the occasional development of triplets. The timing of transformation was also variable; initiation of twinning was observed at all but the earliest stages of seed development. Embryogenic transformation in *twn* occurred only in cells of the suspensor. In more than 2000 mutant seeds examined, no twins were observed arising from other embryonic cell types or maternal tissues.

In addition to polyembryony, a number of other developmental defects were observed in mutant embryos (Fig. 4). Occasionally, young proembryos exhibited aberrant division in the terminal cell, suggesting that the *TWN* gene is active very early in development and may influence the directional control of cell division, a fundamental aspect of plant embryogenesis (Fig. 4A). Older embryos also displayed irregular patterns of cell division, as well as altered morphology and delayed development (Figs. 3C and 4B). Cotyledon defects were common at late stages of development (Fig. 4C). Thus, the *TWN* locus is involved in normal development of the embryo proper, and its disruption can affect a broad range of developmental processes throughout the embryo.

DISCUSSION

We describe here an embryo-defective mutant of *Arabidopsis* that exhibits frequent polyembryony following embryogenic transformation of the suspensor. Polyembryony has been described previously in inbred lines of several crop plants (Green and Salisbury, 1983; Chen *et al.*, 1985; Rowland and Weerasena, 1986), but the underlying genetic causes of twinning in such cases have been complex and difficult to resolve. The polyembryony observed in *twn* occurs at a considerably higher frequency than previously reported examples of twinning. Furthermore, the polyembryony of *twn* appears to be caused by a single pleiotropic mutation that has incomplete penetrance. We therefore propose that *twn* represents the first reported example of frequent and heritable polyembryony in dicotyledonous plants caused by mutation at a single genetic locus.

Twn displays abnormal development in the embryo proper as well as embryogenic transformation of the suspensor. Three models may account for the distinct effects of the *twn* mutation on the embryo proper and suspensor. Two of these models underscore the importance of proper interaction between different parts of the developing embryo. In the first model, *TWN* functions primarily in the suspensor. Defects observed in the embryo proper could result from failure of the suspensor to function properly in developing mutant seeds. This model does not account for the observation that *twn* embryos occasionally exhibit defects in the embryo proper very early in embryogenesis, prior to the appearance of any suspensor abnormalities (Fig. 4A). We propose that *TWN* is active in the embryo proper and is required for proper communication between the embryo proper and suspensor. Abnormal suspensor growth in mutant seeds would then be a consequence of defective

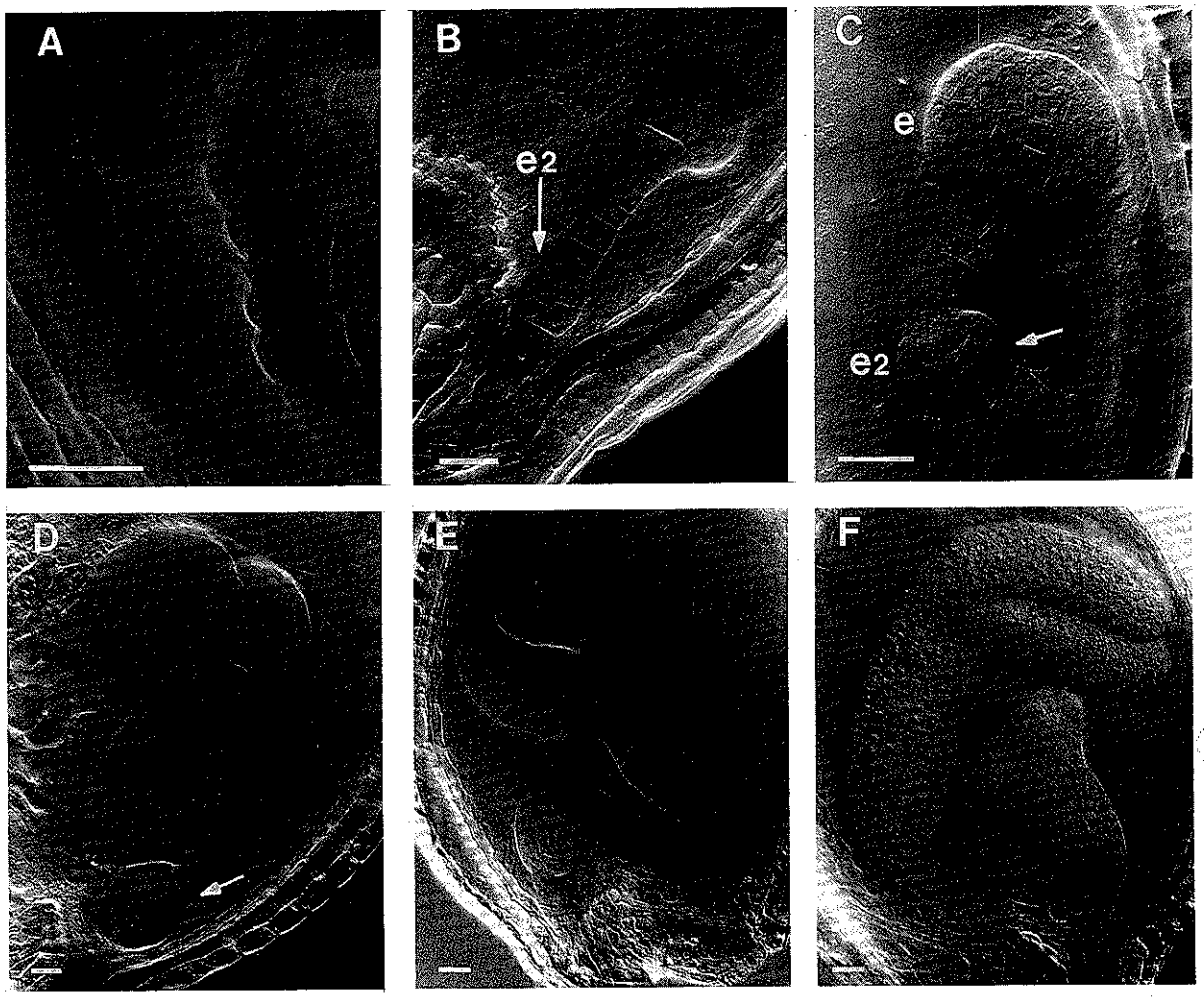

FIG. 3. Origin of supernumerary embryos in developing mutant seeds. (A) Twins are not present in young mutant seeds. The early proembryo shown here resembles wild type: it consists of a terminal cell and an elongate basal cell, the progenitor of the suspensor. (B) Initiation of twinning in a globular stage seed. The cell adjacent to the basal cell of the suspensor has divided to form an octant stage secondary embryo (e2). (C) Secondary embryo within an intact mutant suspensor. The seed is from a heart stage silique. The developing twin (e2) exhibits a clear hypophysis (arrow) and a developing protoderm. The primary embryo (e) is morphologically abnormal. (D) Linear stage embryo accompanied by an oblong secondary embryo. The hypophysis in the secondary embryo (arrow) has formed proximal to the primary embryo, indicating that orientation of this embryo is opposite that of its sibling. (E) Linear stage seed showing the intact suspensor connecting both embryos to the seed coat. (F) Twin pair in a cotyledon stage seed. The secondary embryo has reduced cotyledons. Siblings are no longer linked by the suspensor, although a suspensor segment still connects the younger sibling to the seed coat. Scale bar, 20 μm.

TWN activity in the embryo proper. Such a scenario would be consistent with the wide range of phenotypes observed in this mutant and the traditional view that suspensor growth is inhibited in part by interaction with the embryo proper (Haccius, 1955; Yeung and Meinke, 1993). A similar model has been proposed to explain the enlarged suspensors seen in many *Arabidopsis* embryo-lethal mutants, some of which may be defective in general cellular functions (Marsden and Meinke, 1985; Yeung and Meinke, 1993). The complete embryogenic transformation observed in *twn*, however, distinguishes *twn* from other abnormal suspensor mutants. This unique embryogenic transformation may occur because the *twn* mutation disrupts embryo–suspensor interaction to just the right extent, relieving the inhibitory effect of the embryo proper without consistently preventing secondary embryos from completing development. Alternatively, *TWN* may play a novel, direct role in establishing and maintaining embryo–suspensor communication.

A third model, in which the *TWN* gene is active in both the embryo proper and the suspensor, cannot be ruled out by our observations. This model raises the intriguing possibility that the *TWN* gene product carries out different functions in the embryo proper and the suspensor. Specifically, TWN could be directly involved in the suppression of embryogenic development in the suspensor, while having a more wide-ranging role support-

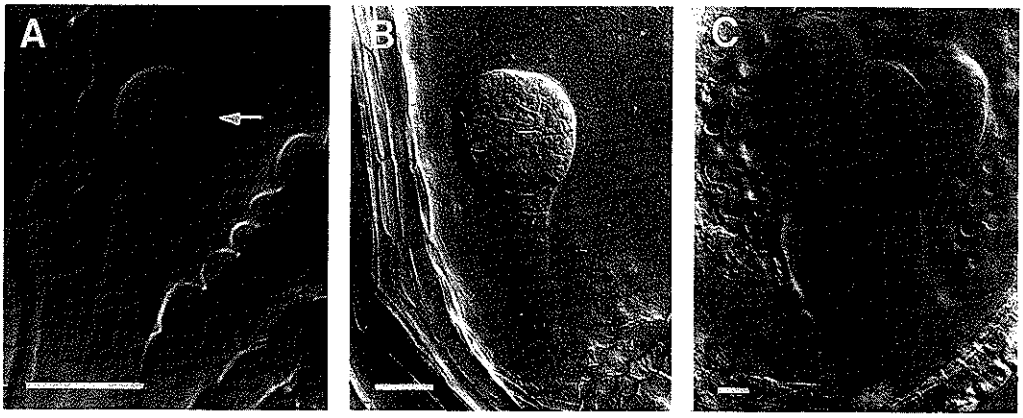

FIG. 4. Additional developmental abnormalities observed in *twn* embryos. (A) Aberrant cell division in the terminal cell of a mutant proembryo. The first division of the embryo proper (arrow) is almost horizontal rather than longitudinal (compare with wild type in Fig. 2A). (B) Defective embryo from a heart stage silique. Patterns of cell division in the embryo proper are irregular, and morphological development of the embryo is defective (compare with wild type in Fig. 2C). (C) Linear stage embryo with three defined cotyledons. Scale bar, 20 μm.

ing continued development of the embryo proper. Thus, mutation at the *TWN* locus would result in both suspensor transformation and the diverse developmental abnormalities observed in the embryo proper. Such a dual role for a single gene product is conceivable if, for example, TWN plays a role in an intercellular or intracellular signaling process that is perceived differently by distinct cell types.

Disruption of the *TWN* locus can result in a broad range of developmental defects in the embryo proper. Despite extensive characterization of *twn* embryo development by Nomarski optics, no single defect was consistently observed in the embryo proper. Such phenotypic diversity is a common feature of embryo-defective mutants (Meinke, 1991b). Indeed, two of the best-characterized *Arabidopsis* embryo mutants, *gnom* (*emb30*) and *bio1*, display a high degree of variability in their morphology and developmental potential (Mayer *et al.*, 1993; Schneider *et al.*, 1989). The diversity of developmental defects observed in *twn* suggests that *TWN* does not regulate one specific feature of embryo development. Rather, *TWN* is likely to be involved in a fundamental process or pathway that can have wide-ranging effects when disrupted. The incomplete penetrance and variable severity of the *twn* mutation may indicate that TWN is not absolutely required for normal embryogenesis, perhaps playing an accessory role to other, more fundamental factors. Alternatively, *twn* may represent a relatively weak allele of a gene crucial for embryogenesis. Either model is consistent with the proposed role for *TWN* in maintaining normal communication between the embryo proper and the suspensor. Indeed, the unique polyembryony observed in *twn* may occur because the incomplete penetrance of the *twn* allele disrupts embryo–suspensor communication while occasionally allowing completion of embryogenesis in transformed suspensors. Elucidation of the exact nature of the *twn* mutation will require cloning and molecular characterization of the *TWN* locus.

The development of viable embryos from cells of the *twn* suspensor is the most intriguing and informative aspect of the mutant phenotype. The polyembryonic phenotype of *twn* demonstrates that cells of the *Arabidopsis* suspensor have the capacity to duplicate the full spectrum of developmental programs usually restricted to the embryo proper. Although *in vitro* studies of somatic embryogenesis have previously demonstrated the totipotent capacity of plant cells, the *twn* phenotype provides a unique example of embryogenic transformation. In *twn*, a heritable genetic defect triggers embryo development from a specific cell type *in vivo*. This transformation occurs in a differentiated structure and appears to follow the developmental patterns characteristic of zygotic embryogenesis. Secondary embryo development in *twn* is also distinct from the "suspensor polyembryony" observed in some species, in which typically inviable proembryos bud from undifferentiated suspensors of developing primary embryos (Johri *et al.*, 1992).

The potential of suspensor cells to duplicate normal embryo development includes the capacity to establish embryonic polarity. This suggests that development of polarity within the seed is not absolutely dependent on asymmetric conditions specific to the zygote or unfertilized egg. Interestingly, sibling embryos within the same seed often developed in opposite orientations (e.g., Fig. 3D). Thus, interaction with surrounding seed tissues does not appear to dictate the orientation of embryonic polarity, as has previously been suggested for embryo development *in ovulo* (Cooke and Cohen, 1993). Rather, polarity appears to be established by programs active within the developing embryo itself.

The polyembryony of *twn* provides a striking example

of cellular transformation. Disruption of *TWN* function results not in disorganized neoplastic growth, but in reversion of a differentiated cell type to an embryogenic state and activation of embryogenic developmental programs. The *twn* phenotype implies that in the *Arabidopsis* suspensor, suppression of embryogenic potential and maintenance of a differentiated state are interrelated processes that can be disrupted, directly or indirectly, by mutation at a single genetic locus. Although cell differentiation is often viewed as an inductive process involving activation of cell-specific genetic programs, the derepression of embryogenic programs that results from loss of *TWN* function suggests an important role for negative regulatory mechanisms in defining cell type. In light of the totipotent nature of plant cells, mechanisms that suppress specific developmental pathways may be of widespread significance for the establishment and maintenance of cell identity during plant development.

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REFERENCES

Akhundova, G. G., Shevchenko, V. V., and Grinikh, L. I. (1979). Twin plants of *Arabidopsis thaliana* induced by gamma irradiation. *Sov. Genet.* **15,** 428–432.

Barton, M. K., and Poethig, R. S. (1993). Formation of the shoot apical meristem in *Arabidopsis thaliana:* An analysis of development in the wild-type and in the *shoot meristemless* mutant. *Development* **119,** 823–831.

Baus, A. D., Franzmann, L., and Meinke, D. W. (1986). Growth *in vitro* of arrested embryos from lethal mutants of *Arabidopsis thaliana*. *Theor. Appl. Genet.* **72,** 577–586.

Berleth, T., and Jürgens, G. (1993). The role of the *monopteros* gene in organizing the basal body region of the *Arabidopsis* embryo. *Development* **118,** 575–587.

Castle, L. A., Errampalli, D., Atherton, T., Franzmann, L., Yoon, E., and Meinke, D. (1993). Genetic and molecular characterization of embryonic mutants identified following seed transformation in *Arabidopsis*. *Mol. Gen. Genet.* **241,** 504–514.

Chen, L. O., Heer, H. E., and Palmer, R. G. (1985). The frequency of polyembryonic seedlings and polyploids from ms_1 soybean. *Theor. Appl. Genet.* **69,** 271–277.

Clark, J. K., and Sheridan, W. F. (1991). Isolation and characterization of 51 embryo-specific mutations of maize. *Plant Cell* **3,** 935–951.

Cooke, T. J., and Cohen, J. D. (1993). The role of auxin in plant embryogenesis. *Plant Cell* **5,** 1494–1495.

Errampalli, D., Patton, D., Castle, L., Mickelson, L., Hansen, K., Schnall, J., Feldmann, K., and Meinke, D. (1991). Embryonic lethals and T-DNA insertional mutagenesis in *Arabidopsis*. *Plant Cell* **3,** 149–157.

Feldmann, K. A. (1991). T-DNA insertion mutagenesis in *Arabidopsis:* Mutational spectrum. *Plant J.* **1,** 71–82.

Green, A. G., and Salisbury, P. A. (1983). Inheritance of polyembryony in flax. *Can. J. Genet. Cytol.* **25,** 117–121.

Haccius, B. (1955). Experimentally-induced twinning in plants. *Nature* **176,** 355–356.

Haccius, B. (1963). Restitution in acidity-damaged plant embryos: Regeneration or regulation? *Phytomorphology* **13,** 107–115.

Johri, B. M., Ambegaokar, K. B., and Srivastava, P. S. (1992). "Comparative Embryology of Angiosperms." Springer-Verlag, Berlin.

Keith, K., Kraml, M., Dengler, N. G., and McCourt, P. (1994). *fusca3*: A heterochronic mutation affecting late embryo development in *Arabidopsis*. *Plant Cell* **6,** 589–600.

Mansfield, S. G., and Briarty, L. G. (1991). Early embryogenesis in *Arabidopsis thaliana*. II. The developing embryo. *Can. J. Bot.* **69,** 461–476.

Marsden, M. P. F., and Meinke, D. W. (1985). Abnormal development of the suspensor in an embryo-lethal mutant of *Arabidopsis thaliana*. *Am. J. Bot.* **72,** 1801–1812.

Mayer, U., Torres Ruiz, R. A., Berleth, T., Miséra, S., and Jürgens, G. (1991). Mutations affecting body organization in the *Arabidopsis* embryo. *Nature* **353,** 402–407.

Mayer, U., Buttner, G., and Jürgens, G. (1993). Apical-basal pattern formation in the *Arabidopsis* embryo: Studies on the role of the *gnom* gene. *Development* **117,** 149–162.

Meinke, D. W. (1991a). Perspectives on genetic analysis of plant embryogenesis. *Plant Cell* **3,** 857–866.

Meinke, D. W. (1991b). Embryonic mutants of *Arabidopsis thaliana*. *Dev. Genet.* **12,** 382–392.

Meinke, D. W. (1992). A homoeotic mutant of *Arabidopsis* with leafy cotyledons. *Science* **258,** 1647–1650.

Meinke, D. W. (1994). Seed Development in *Arabidopsis*. *In "Arabidopsis"* (E. Meyerowitz and C. Somerville, Eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, in press.

Meinke, D. W., Franzmann, L. H., Nickle, T. C., and Yeung, E. C. (1994). *Leafy cotyledon* mutants of *Arabidopsis*. *Plant Cell* **6,** in press.

Murashige, T., and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* **15,** 493–497.

Patton, D. A., Franzmann, L. H., and Meinke, D. W. (1991). Mapping genes essential for embryo development in *Arabidopsis thaliana*. *Mol. Gen. Genet.* **227,** 337–347.

Rowland, G. G., and Weerasena, L. A. (1986). Some observations on polyembryony in crosses between twinning and non-twinning lines of flax. *Can. J. Plant Sci.* **66,** 819–824.

Schneider, T., Dinkins, R., Robinson, K., Shellhammer, J., and Meinke, D. W. (1989). An embryo-lethal mutant of *Arabidopsis thaliana* is a biotin auxotroph. *Dev. Biol.* **131,** 161–167.

Tisserat, D. W., Esan, E. B., and Murashige, T. (1979). Somatic embryogenesis in angiosperms. *Hort. Rev.* **1,** 1–78.

Yeung, E. C., and Meinke, D. W. (1993). Embryogenesis in angiosperms: Development of the suspensor. *Plant Cell* **5,** 1371–1381.