Mutations in the PILZ group genes disrupt the microtubule cytoskeleton and uncouple cell cycle progression from cell division in Arabidopsis embryo and endosperm

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Organised cell division and expansion play important roles in plant embryogenesis. To address their cellular basis, we have analysed Arabidopsis abnormal-embryo mutants which were isolated for their characteristic phenotype: mutant embryos are small, mushroom-shaped ("pilz") and consist of only one or few large cells each containing one or more variably enlarged nuclei and often cell wall stubs. These 23 mutants represent four genes, PFIPPERLING, HALLIMASCH, CHAMPIGNON, and PORCINO, which map to different chromosomes. All four genes have very similar mutant phenotypes although porcino embryos often consisted of only one large cell. The endosperm did not celluarise and contained a variably reduced number of highly enlarged nuclei. By contrast, genetic evidence suggests that these genes are not required for gametophyte development. Expression of cell cycle genes, Cdk2a, CyclinA2 and CyclinB1, and the cytokinesis-specific KNOLLE gene was not altered in mutant embryos. However, KNOLLE syntaxin accumulated in patches but no KNOLLE-positive structure resembling a forming cell plate occurred in mitotic cells. A general defect in microtubule assembly was observed in all mutants. Interphase cells lacked cortical microtubules, and spindles were absent from mitotic nuclei although in rare cases, short stubs of microtubules were attached to partially condensed chromosomes. Our results suggest that the cellular components affected by the pilz group mutations are necessary for continuous microtubule organisation, mitotic division and cytokinesis but do not mediate cell cycle progression.

Introduction

Plant cells are encased in cell walls, which prevents them from moving as animal cells do. As a consequence, morphogenetic processes that shape the developing plant embryo involve organised cell division and oriented cell expansion, in addition to cell-cell communication (for review, see [12]). Because organised cell division and oriented cell expansion are developmentally integrated, underlying cellular mechanisms may be revealed from the analysis of abnormal-embryo mutants, focusing on cytokinesis, cell cycle regulation and the microtubule cytoskeleton.

Cytokinesis partitions the cytoplasm of the dividing cell, creating separate microenvironments for the daughter nuclei which can thus receive different signals from their neighbours. For example, cytokinesis is severely impaired in Arabidopsis knolle embryos, and the LTP gene whose expression is normally confined to the epidermis primordium is activated ubiquitously in the young embryo [37]. The KNOLLE gene encodes a cytokinesis-specific syntaxin and is transcriptionally regulated in a cell cycle-dependent manner [16]. The KNOLLE protein accumulates during mitosis and is then relocated to the plane of cell division where it mediates cell plate formation by vesicle fusion [13].

Cell cycle-regulated expression of components required for proper execution of cytokinesis indicates that the two processes are closely linked. Molecular components of the cell-cycle machinery, such as Cdk2 kinases (cyclin-dependent kinases, CDKs), cyclins and CDK inhibitors, have been identified in higher plants (for review, see [3]). The Cdc2a gene is expressed in division-competent cells of Arabidopsis plants whereas mitotic (B-type) cyclin CycB1;1 transcripts accumulate in actively dividing cells [28]. Transgenic Arabidopsis plants expressing the CycB1;1 cDNA from the Cdc2a pro-
motors show accelerated root growth due to increased cell division rate but no indication of altered root organisation [4]. Transgenic tobacco plants expressing a dominant negative form of the *Arabidopsis* Cdc2a kinase display a dwarf phenotype and predominantly consist of enlarged cells whereas transgenic *Arabidopsis* plants bearing the same construct could not be recovered [10], presumably due to a block in cell division causing embryo lethality.

Plant microtubules have been implicated in organising cell division and cell expansion. They undergo reorganisation in a cell cycle-dependent manner (for review, see [6]). During interphase, cortical microtubules are aligned perpendicular to the long axis of the cell, thus delimiting the direction of cell expansion. Mitotic cells display a succession of specific microtubule arrays each of which associates with Cdc2a kinase [31]. At the transition from G2 to M phase, a cortical preprophase band containing microtubules presages the orientation of cell division. The mitotic spindle is involved in the alignment of chromosomes at metaphase and their movement to opposite poles during anaphase. The phragmoplasts assemble in cell plate formation during cytokinesis, mediating vesicle trafficking to the plane of cell division (for review, see [30]). While the role of the microtubule cytoskeleton in plant cell division has been intensively studied in specific experimental systems, such as tobacco BY-2 cells and *Trisectancia* stamen hair cells, the functional role of microtubules in *Arabidopsis* development has been scarcely addressed (for review, see [29]). One relevant class of developmental mutants has been described. In *Arabidopsis fass* and *tonneau* mutants, both cell expansion and cell division are disorganised from early embryogenesis, resulting in severely stunted adult plants with proximo-distally compressed organs [20, 34, 35]. This abnormal morphogenesis correlates with disorganised cortical arrays of interphase microtubules and the absence of preprophase band microtubules [20, 35]. I. Weinegger and U. Mayer, unpublished observation). By contrast, the mitotic spindle and the phragmoplast are not affected in the mutants.

In a search for *Arabidopsis* mutations affecting cytokinesis during embryo development, we have isolated mutants that perform no or only a few cell divisions. Here, we analyse the cellular basis for this developmental defect. Our results suggest that mutant cells continue to cycle but do not complete mitosis, fail to execute cytokinesis and are defective in the assembly of microtubule arrays.

**Material and methods**

**Plant strains, growth conditions, genetic methods**

Landsberg *erecta* (Ler) was used as wild-type control, Columbia (Col) for mapping of mutants. Transgenic *Arabidopsis* plants bearing Cdc2a-GUS [9], CycB1;1-GUS [28], Bursseens et al., submitted) or CycB1;1-GUS [5] constructs were crossed with *plit* group mutants, their F1 progeny were selected for heterozygosity, and F2 ovules were stained for GUS expression. Plants were grown on soil under continuous illumination at 18°C or 25°C as previously described [19].

*Ler* seeds were mutagenised with 0.3% EMS for 8h as previously described [18]. The main shoot of M1 plants was cut off, and seeds were harvested from a side shoot because the spongy tissue of a side shoot originates from a single mutagenised cell in about 95% of the cases [22]. Two M2 plants each were grown from 1500 lines, one or two siliques were removed from each plant, the ovules were mounted in chloralhydrate and inspected microscopically for abnormal embryos [19].

Complementation tests which were performed as previously described [18] gave the following complementation groups: *Pffferling* (PFI): UU685, UU996, UU2221, UU2245, UU2943, UU2975; *Hallimasch* (HAL): UU2013, UU2339, UU2410; *Champignon* (CHO): UU404, UU908, UU1091, UU1098, UU1159, UU1257, UU1424, UU1833, UU2279, UU3592; *Porcion* (POR): UU1087, UU3025. Additional *plit* (ML375) and *cho* (ML479) alleles were recovered from an X-ray mutagenesis (20000 R) of *Ler* seeds (U. Mayer, unpublished observation).

For mapping of *plit* group mutants with molecular markers, one allele each of the four complementation groups was crossed with *Col*. Heterozygous *plit* F1 plants were selected and selfed to produce F2 mapping populations. DNA was CTAB-extracted from leaves of wild-type and heterozygous F2 plants from each cross and PCR-amplified with sequence-specific primer pairs; the amplified DNA fragments were restriction-digested to reveal polymorphisms if necessary (CAPS markers) and separated on agarose gels as previously described [16]. The following SSLP [2] and CAPS markers were used (KI map positions in brackets; http://nasc.nott.ac.uk/new.rfl.map.html): nga 63 (1-9), nga111 (1-111), vtol2 (2-7), nga68 (2-73), nga126 (3-16), nga66 (3-35), nga8 (4-24), g435 (4-55), nga151 (5-29), nga76 (5-72). The primers vtol2 and g435 were based on sequence information from D. Bouchez, INRA Versailles (H. Illgenfritz, personal communication) and the CAPS marker web site (http://genome-www.stanford.edu/Arabidopsis/aboutcaps.html), respectively.

**Results**

**Genetic characterisation of *plit* mutants**

A screen for abnormal-embryo phenotypes yielded 23 lines that produced small mushroom-shaped (*PLIT*) embryos upon selfing of heterozygous plants. When the wild-type siblings from the same siliques were approaching the mature-embryo stage the mutant embryos were still alive but had failed to accumulate chlorophyll, which is normally initiated at the late-heart stage. The mutant embryos consisted of one or a few enlarged cells each with one to several nuclei and were surrounded by non-cellularised endosperm which contained fewer and larger nuclei than wild-type endosperm at cellularisation (Fig. 1).

The *plit* mutants were shown by complementation tests to represent four different genes, *Champignon* (CHO), *Hallimasch* (HAL), *Pffferling* (PFI) and *Porcion* (POR), comprising between 2 and 11 alleles (Table I). Each gene was PCR-mapped against molecular markers anchored on the recombinant inbred map [14]. Linkage analysis placed each gene in a specific region on a different chromosome (Table I). Heterozygous plants from the mapping populations produced nearly the expected proportion of heterozygous progeny, suggesting that mutations in these genes do not
Fig. 1. Phenotypes of pili embryos and endosperm. (A) pfi-UU996 (E; bottom) and wild-type early-torpedo (E; top) embryos from same silique. Note large endosperm nuclei (ES) in pfi ovule. (B) pfi-UU2914 young embryo (E) and several large endosperm nuclei (ES). (C) hal-UU2013 (top) and wild-type heart-stage (bottom) embryos from same silique. (D) hal-UU2410 old embryo consisting of 2 large cells. (E) cho-UU404 old embryo consisting of 4-6 large cells. (F) por-UU1087 old embryo consisting of 1 large cell. Whole-mount preparations. Nomarski optics.

affect gametophyte development (Table 1). There were no obvious phenotypic differences between alleles of the same gene which would enable alleles to be classified as weak or strong. Mutations in different genes also shared common phenotypic features although por mutations appeared to confer a stronger phenotype than mutations in the other genes. Many por embryos were single-celled, containing one to a few enlarged nuclei, while pfi, hal and cho embryos consisted of

Tab. 1. Genetic characteristics of PILZ group genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Map position</th>
<th>Linked marker</th>
<th>Rec/chrom. (cM)</th>
<th>No. of alleles</th>
<th>% Tr. (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAMPIGNON (CHO)</td>
<td>3-90 ±</td>
<td>nge6</td>
<td>2/68 (3 cM)</td>
<td>11</td>
<td>65 (113)</td>
</tr>
<tr>
<td>HALLIMASCH (HAL)</td>
<td>2-40 ±</td>
<td>va012</td>
<td>30/122 (35 cM)</td>
<td>3</td>
<td>60 (231)</td>
</tr>
<tr>
<td>PFIFTERLING (PFI)</td>
<td>1-110 ±</td>
<td>nga111</td>
<td>5/132 (4 cM)</td>
<td>7</td>
<td>66 (340)</td>
</tr>
<tr>
<td>PORCINO (POR)</td>
<td>4-90 ±</td>
<td>g4539</td>
<td>21/106 (25 cM)</td>
<td>2</td>
<td>63 (303)</td>
</tr>
</tbody>
</table>

a Approximate value estimated from number of recombinants with linked marker. b Proportion of recombinant chromosomes among all chromosomes analysed; distance (cM) as calculated from recombination frequency, using mapping function. c Transmission frequency of mutant allele in selfing of heterozygous plants (expected value is 67% if transmission is normal); N, number of progeny analysed. Alleles studied: cho-UU1159, hal-UU2013, pfi-UU686, por-UU1087.
Expression of cell-cycle and cytokinesis genes in pilz group mutant embryos

To address how the cell division defects come about, we analysed the expression of genes involved in cell cycle regulation and cytokinesis. Misregulation of genes that encode components of cyclin-dependent kinase (CDK) complexes would be a good criterion by which to assess whether the cell cycle is altered or arrested in mutant embryos. The a-type kinase gene Cdc2a is constitutively expressed in division-competent cells [3]. Two mitosis-specific cyclin genes, CycA2 and CycB1, are expressed in actively dividing cells. CycB1 is normally expressed from the beginning of S phase until metaphase whereas maximal expression of CycB1 is observed during the G2 and M phases [3]. We analysed GUS activity resulting from reporter gene expression driven by each promoter to determine whether the cells of advanced-stage mutant embryos were competent to divide and were progressing through the cell cycle, respectively. GUS staining was observed in wild-type and mutant embryos for all three reporter gene fusions, suggesting that mutant cells remained division-competent and had progressed through the cell cycle (Fig. 3). To exclude that stability of the GUS protein might have masked any regulatory defect in the mutant embryos, we also performed in situ hybridisation with a CycB1 probe. Wild-type embryos displayed a patchy pattern of expression, with only some cells accumulating CycB1 mRNA (Fig. 4A). The same result was obtained for few-celled mutant embryos, confirming data obtained by GUS staining (Fig. 4A, B). Thus, individual cells of few-celled embryos cycled independently, regardless of the number of nuclei. Collectively, the study of Cdc2a and cyclin gene expression suggests that the cell cycle is not arrested in the pilz group mutants.

two to five cells each with one or a few nuclei (Fig. 1, 2). The shape of mutant embryos was somewhat variable, even if the embryo was a single cell. Both single-celled and few-celled mutant embryos were often basally constricted and apically widened. Other single-celled embryos were ellipsoidal in shape while few-celled embryos appeared apically lobed, due to short common walls between grossly constricted cells (Fig. 1E, F; 2A, C). The basal tube-like portion of few-celled embryos consisted of two or three cells or contained several nuclei along the apical-basal axis, thus resembling the suspensor in normal seed development. Grossly enlarged nuclei contained large nucleoli and were often abnormally-shaped in both embryo and endosperm (Fig. 2C, D; see Fig. 7D, H). Mitotic chromosomes were observed but neither metaphase plates nor anaphase or telophase configurations (see Fig. 5, 6, and data not shown).

Young mutant embryos consisted of a similar number of cells as older mutant embryos (Fig. 1A, B; 2A, B). However, the cells of young embryos were comparatively normal in size and shape, and the nuclei were only slightly enlarged, suggesting that subsequent development of mutant embryos predominately increased cell and nuclear size and, possibly, number of nuclei. The number of endosperm nuclei was similar in young and old ovules but their size decreased during development. In both embryo and endosperm, the enlarging nuclei and nucleoli also attained an abnormal shape.

Electron microscopy of pf, cho and por embryos confirmed the convoluted shape of nuclei and also revealed the presence of cell wall stubs and internal cell wall fragments (Fig. 2C–F). Cells with variably enlarged multiple nuclei and cell wall stubs have previously been noted in Arabidopsis cytokinesis-defective mutants, such as knolle [16] and keule [1]. In contrast to the latter mutants, both cell wall stubs and internal wall fragments were often abnormally shaped and appeared fuzzy as if cell wall material had been deposited in a sphere rather than a plane (Fig. 2F). In summary, the pilz group mutations appear to affect mitosis and cytokinesis.

In the following experiments we analysed the cellular phenotypes of pilz group mutants with several molecular markers, studying at least one allele per gene. For space limitations, only a representative selection of the results is documented in the figures. If not otherwise stated, no obvious differences were observed between pilz group mutants.
The KNOLLE gene encodes a cytokinesis-specific syntaxin and gives a patchy expression pattern in wild-type embryos, very much like CycB1 expression [16]. Mutant embryos also showed a patchy pattern, with one of several cells accumulating the KNOLLE mRNA (Fig. 4C). This result indicates that mutant cells respond to the signal for cell division but apparently fail to execute cytokinesis. In wild-type embryo cells, KNOLLE protein accumulates in large cytoplasmic patches during mitosis and is relocated to the plane of cell division during cytokinesis (Fig. 5A, B; [13]). We observed KNOLLE-positive patches in mitotic cells of mutant embryos but no KNOLLE-positive structure resembling a cell plate was formed (Fig. 5C, D). All mutants gave similar results although aggregates of KNOLLE-positive material were sometimes larger (data not shown). Thus, mitotic cells of mutant embryos fail to organise the formation of well-formed cell plates.
Analysis of the microtubule cytoskeleton in mutant embryos

Since microtubules assist in the cellular processes that appeared to be affected in mutant embryos we used DAPI and α-tubulin antibody staining to monitor cell cycle-dependent microtubule arrays. Regardless of the mutated gene, all pilz group embryos showed essentially the same defects (Fig. 6). Neither interphase nor mitotic cells displayed any of the characteristic microtubule arrays. In most cases, there was no specific antibody staining in mutant embryos whereas the surrounding maternal tissue of the ovule gave bright signals (Fig. 6F, H, J, L). In some cases, short thick stubs of microtubules were associated with numerous mitotic chromosomes (Fig. 6C, D). DAPI staining revealed large groups of chromosomes which, however, were not aligned in metaphase plates, nor did we observe anaphase or telophase configurations (Fig. 6C, E, G). In large cells chromosome numbers exceeded the wild-type complement manifold (for example, see Fig. 6C). Our results suggest that pilz group mutant embryos are impaired in the formation of microtubules.

The microtubule cytoskeleton in mutant endosperm

Before cellularisation, endosperm nuclei cycle synchronously [17]. Microtubule asters radiate from the surface of interphase nuclei in wild-type early endosperm development (Fig. 7A, E). By contrast, no distinct α-tubulin staining was observed in the cytoplasm surrounding enlarged interphase nuclei of pilz group mutants (Fig. 7B, F). During wild-type endosperm mitosis, well-formed mitotic spindles associate with the chromosomes and pull the two sets of daughter chromosomes apart (Fig. 7C). In pilz group mutants, the mitotic chromosomes were not separated into daughter sets and were associated with short thick stubs of microtubules which did not clon-
gate to the wild-type length (Fig. 7G). These results suggest that the mutant endosperm lacks a necessary condition for microtubule organisation.

**Discussion**

The *Arabidopsis* embryo displays a highly ordered pattern of cell division and oriented cell expansion and is thus well suited for a genetic analysis of mechanisms that underlie these cellular processes. We have identified mutations in four genes collectively called the PILZ group which give very similar phenotypes: the mutant embryos consist of one or a few enlarged cells. Further analysis suggests that the cell division defect is not associated with a cell cycle arrest but correlates with a loss of microtubule arrays. This combination of features is unique among *Arabidopsis* embryo-defective mutants analysed so far. *fass* and *tonneau* mutants display altered cell division planes and abnormal cell shapes which correlate with the absence of the preprophase band and abnormal interphase cortical microtubules [20, 35]. These cellular defects, however, do not cause embryo lethality but result in stunted seedlings and adult plants [34, 35]. A different class of cell division abnormalities is observed in cytokinesis-defective embryo mutants of pea (*cyl* [15]) and *Arabidopsis* (*knolle* [16], *keule* [1], *cyl* [23]). So far, only the KNOLLE gene has been isolated and shown to encode a cytokinesis-specific syntaxin which accumulates in the plane of division during cell plate formation [13, 16]. As shown here, *pilz* group mutants fail to form a KNOLLE-positive cell plate and lack phragmoplast microtubules along which cytokinetic vesicles are normally transported [36]. By contrast, *knolle* mutant cells which are specifically impaired in cytokinesis accumulate unfused membrane vesicles in the plane of cell division [13] and form phragmoplast microtubules (I. Wüzenegger, G. Jürgens, and U. Mayer, unpublished observation). In the following we discuss how the cell division defect of *pilz* group mutants may be related to the cell cycle and the microtubule cytoskeleton.

Cell division is normally triggered by active CDKs associated with cyclin B. Active Cdc2/cyclin B complex causes nuclear envelope breakdown and rapid disassembly of the preprophase band when injected into *Trailing* stamen hairs [11]. Various isoforms of maize B-cyclins [21] and the *Arabidopsis* Cdc2a kinase [31] have been immuno-localised to preprophase band, mitotic spindle and phragmoplast. Overexpression and antisense expression of cyclin B have opposite effects on cell division in both *Arabidopsis* and tobacco transgenic plants [4, 10], indicating that cyclin B plays an important regulatory role in cell division control. The expression of CDK components was not obviously affected in *pilz* group mutants, and individual cells of mutant embryos accumulated cyclin B mRNA and KNOLLE mRNA independently of their neighbours, suggesting that the cell cycle-dependent transcriptional regulation of these genes is effective. Furthermore, the cytokinesis-specific KNOLLE syntaxin accumulated in mitotic cells but not in interphase cells of mutant embryos, which is to be expected if KNOLLE protein disappeared at the M/G1 transition as it does in wild-type cells [13]. Thus, *pilz* group mutants appear to progress through the cell cycle. However,
mitotic cells did not undergo the normal sequence of M-phase events. Although clusters of chromosomes were frequently found, no metaphase, anaphase or telophase figures nor an alignment of KNOX-like-positive material corresponding to a forming cell plate were observed. This suggests that M phase progression is blocked before metaphase and that mutant cells then re-enter interphase without prior cytokinesis.

Passing through mitosis and executing cytokinesis requires a number of structural and functional features of the dividing plant cell, such as chromosome condensation, alignment and movement, vesicle trafficking and fusion to form the cell plate, and reorganisation of the microtubule and actin cytoskeleton [29, 30]. These processes involve interacting components such that absence or inactivity of one or another component may result in aborted cell division. In higher plants, little is known about mechanisms underlying the orderly sequence of events by which the chromosomes are faithfully segregated to the daughter cells. By contrast, mitotic checkpoints have been identified in yeast and Drosophila by mutations that result in abnormal M/G1 transition. In fission yeast cut mutants, for example, cytokinesis occurs in the absence of normal sister-chromatid separation [38]. The cut gene products are involved in various mitotic processes, such as chromatin condensation, sister-chromatid separation or metaphase-anaphase progression. Several mutations affect mitotic sister-chromatid separation or spindle attachment in Drosophila [24]. For example, pimmon mutant cells, which do not separate the sister chromatids in the centromeric region, fail to undergo cytokinesis and re-enter interphase [32]. By analogy, the pilz group mutants may identify a mitotic checkpoint that normally ensures orderly progression through M phase and execution of cytokinesis. If this is the case a defect in the presumed checkpoint would have to result in the eventual loss of microtubule arrays.

The absence of microtubule arrays could in principle account for the cellular abnormalities seen in pilz group mutant embryos. For example, the lack of phragmoplast microtubules would be consistent with a failure of forming a KNOX-like-positive cell plate. As a result of disorganised cytokinesis, rather diffuse cell wall stubs and internal wall fragments are made that differ in appearance from the straight cell walls are observed in the cytokinesis-specific mutants knolle [16] and keule [1]. Absence of the mitotic spindle would be consistent with a failure of pilz group mutant cells to align their chromosomes in metaphase plates and move them to opposite poles. The microtubule-depolymerising drug oryzalin has been shown to block mitotic progression of tobacco BY-2 cells before metaphase plates become apparent [28]. In some cases, the mitotic chromosomes of pilz group mutants were associated with short stubs of microtubules. It is noteworthy that in Xenopus extracts, microtubules can self-organise around DNA-coated beads, suggesting that chromatin nucleates mitotic spindles [7]. A spindle-nucleating role has also been inferred for plant chromatin from the observation that a plant antigen recognised by a monoclonal antibody against calf-thymus centromeres associates with the centromeres when the nuclear envelope breaks down during prometaphase [27]. Thus, the loss of microtubule arrays in pilz group mutants may reflect defects in microtubule formation and/or stability.

Drug studies may not reveal the full range of cellular defects resulting from a loss of the microtubular cytoskeleton. Cellular phenotypes caused by mutations in tubulin genes have not been described in higher plants, in contrast to yeast which have only a single β-tubulin gene. In fission yeast, temperature-sensitive β-tubulin mutant cells shifted to the restrictive temperature lose microtubule structures, display abnormal cell shape, execute aberrant cytokinesis and lose viability [25]. Similar cellular defects were also noted in tubulin-defective budding yeast cells [26]. Unfortunately, temperature-sensitive alleles of PILZ group genes are not available for comparable studies of cultured Arabidopsis cells to determine whether the loss of microtubules precedes or follows the appearance of cell division defects. Another approach to study the relationship between loss of microtubules and cell division defect would be to analyse very early embryogenesis of pilz group mutants. However, mutant embryos can only reliably be distinguished from wild-type embryos after they have become visibly abnormal, and the small size of very young embryos renders this analysis currently not feasible. Thus, there is at present no way to distinguish between the two possible scenarios of how mutations in PILZ group genes may result in the cell division defect: loss of microtubule organisation or mitotic checkpoint defect.

In summary, we have identified multiple alleles of four genes with a characteristic cell division defect in a large-scale mutagenesis that nearly reached saturation. This makes it unlikely that several other phenotypically related genes exist in the Arabidopsis genome and were missed by chance. Our analysis suggests that the PILZ group gene functions may be involved in the same pathway or converge on the same cellular process required to sustain both cell division and microtubule arrays but not cell cycle progression. Isolation and molecular analysis of the PILZ group genes should help to determine their roles in embryo and endosperm development more precisely.

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Note added in proof: Three Arabidopsis mutants, titan1 to titan3, showing grossly enlarged endosperm nuclei have been described by Liu and Meinke (1998), Plant J. 16, 21–31. titan1 also shows an embryo phenotype similar to pilz group mutants.

References


