

The *titan* mutants of *Arabidopsis* are disrupted in mitosis and cell cycle control during seed development

Chun-ming Liu and David W. Meinke*

Department of Botany, Oklahoma State University,
Stillwater, Oklahoma 74078, USA

Summary

We describe in this report a novel class of mutants that should facilitate the identification of genes required for progression through the mitotic cell cycle during seed development in angiosperms. Three non-allelic *titan* (*ttn*) mutants with related but distinct phenotypes are characterized. The common feature among these mutants is that endosperm nuclei become greatly enlarged and highly polyploid. The mutant embryo is composed of a few giant cells in *ttn1*, several small cells in *ttn2*, and produces a normal plant in *ttn3*. Condensed chromosomes arrested at prophase of mitosis are found in the free nuclear endosperm of *ttn1* and *ttn2* seeds. Large mitotic figures with excessive numbers of chromosomes are visible in *ttn3* endosperm. The *ttn1* mutation appears to disrupt cytoskeletal organization because endosperm nuclei fail to migrate to the chalazal end of the seed. How double fertilization leads to the establishment of distinct patterns of mitosis and cytokinesis in the embryo and endosperm is a central question in plant reproductive biology. Molecular isolation of *TITAN* genes should help to answer this question, as well as related issues concerning cell cycle regulation, chromosome movement and endosperm identity in angiosperms.

Introduction

Throughout growth and development, eukaryotic organisms must co-ordinate DNA synthesis, chromosome segregation, and cell division in order to generate differentiated cells with the proper complement of chromosomes. The molecular mechanisms responsible for regulating this mitotic cell cycle have been examined in detail (Edgar and Lehner, 1996; Forsburg and Nurse, 1991; Jacobs, 1995; Stillman, 1996). Cyclin-dependent protein kinases (CDKs) have emerged as important components of a conserved regulatory complex that monitors cell division in eukaryotes (Nasmyth, 1996a). A single CDK functions throughout the cell cycle in yeasts; multiple CDKs are present in multicellular eukaryotes (Pines, 1994; Shaul *et al.*, 1996).

Activity of these protein kinases is determined in part by their association with regulatory proteins (cyclins) whose levels fluctuate throughout the cell cycle (Nurse, 1994). Mitotic cyclins accumulate following the completion of DNA synthesis and trigger the onset of mitosis. Other cyclins appear after mitosis and play a role in the initiation of DNA synthesis. Fluctuations in cyclin concentrations are modulated in part by protein degradation through ubiquitin pathways (King *et al.*, 1996).

Mitosis and cytokinesis are the major cytological events associated with progression through the cell cycle. Successful completion of these events requires integration of elaborate changes in chromosome structure and cytoskeletal organization. Following the completion of DNA synthesis, replicated chromosomes must undergo extensive condensation, become attached to a spindle apparatus in the correct plane of division, delay chromatid separation until the metaphase–anaphase boundary, and move to opposite poles before uncoiling and resuming interphase functions. The molecular mechanisms underlying these cytological changes involve proteins that function in chromosome condensation (Koshland and Strunnikov, 1996); sister chromatid cohesion (Miyazaki and Orr-Weaver, 1994); assembly of the spindle apparatus (Marc, 1997); kinetochore dynamics (Gorbsky, 1995); chromosome movement (Asada and Collings, 1997; Barton and Goldstein, 1996); and cytokinesis (Chang and Nurse, 1996; Staehelin and Hepler, 1996). Details have begun to emerge on how these proteins act in concert to ensure that chromosome condensation, segregation and cytokinesis are co-ordinated to minimize the chance of errors during cell division.

Mutants have played an important role in dissecting the complex network of proteins that function in mitosis and cytokinesis and regulate progression through the cell cycle in model eukaryotes. The most extensive studies have dealt with *Drosophila melanogaster* (Edgar and Lehner, 1996), the fission yeast *Schizosaccharomyces pombe* (Stern and Nurse, 1996), and the budding yeast *Saccharomyces cerevisiae* (Nasmyth, 1996b). The *cdc2* gene of *S. pombe*, which is homologous to *cdc28* in *S. cerevisiae* and *Dmcdc2* in *Drosophila*, was identified by screening for mutants defective in cell division and encodes the principal CDK that regulates the yeast cell cycle. Genetic screens for cell division mutants in *S. pombe* have resulted in the identification of numerous genes required for septum formation and co-ordination of mitosis with cytokinesis (Chang and Nurse, 1996; Simanis, 1995). Terminal phenotypes of these mutants include multinucleate cells without a division septum, cells with incomplete, disorganized or

Received 30 April 1998; revised 23 July 1998; accepted 5 August 1998.

*For correspondence (fax +1 405 744 7074;
e-mail meinke@osuunx.ucc.okstate.edu).

multiple septa, and torn (*cut*) cells in which cytokinesis occurs despite a failure to complete mitosis.

Mutants have not played a central role in the analysis of mitotic cell cycles in plants. Recessive mutations that disrupt mitosis and cell cycle regulation in plants should result in lethality early in development if the altered protein has little residual activity and if duplicate genes and stored maternal products are unable to rescue mutant cells. In contrast to *Drosophila*, where male gametes are not active in transcription and the egg contains stored maternal products that regulate embryo development, plant gametophytes and embryos are active in transcription and likely to regulate their own division. Gametophytic mutants have been analyzed in a variety of angiosperms but progress towards the identification of mutants defective in known regulators of the cell cycle has been limited. Emphasis has been placed instead on descriptions of gametophytic mutants disrupted in cytoskeletal organization and chromosome behavior (Christensen *et al.*, 1997; Huang and Sheridan, 1996; Peirson *et al.*, 1997; Sheridan and Huang, 1997; Staiger and Cande, 1990, 1992) and mutants with defects in cytokinesis during pollen development (Chen and McCormick, 1996; Hülkamp *et al.*, 1997; Spielman *et al.*, 1997).

Embryo-defective mutants represent a vast alternative source of genes with essential functions during plant growth and development (Meinke, 1995). Some of these mutants are likely to be defective in genes required for the completion of cell division. Examples include cytokinesis-defective mutants of pea (Liu *et al.*, 1995b) and *Arabidopsis* (Assaad *et al.*, 1996; Lukowitz *et al.*, 1996; Nickle and Meinke, 1998), which produce embryos with incomplete cell walls, and the *fass* (*tonneau*) mutant of *Arabidopsis*, which lacks microtubular preprophase bands (McClinton and Sung, 1997; Traas *et al.*, 1995). Mutants disrupted in cytokinesis have also been identified by screening for abnormalities later in vegetative development (Liu *et al.*, 1997).

We describe in this report a novel class of *Arabidopsis* mutants defective in mitosis and cell cycle control during seed development. The common feature among these *titan* (*ttn*) mutants is dramatic enlargement of endosperm nuclei early in development. Although mutants with enlarged nuclei have been described in other organisms, the *ttn* mutants described here provide the most striking example of increased nuclear volume resulting from a recessive mutation. The severity of this mutant phenotype demonstrates that *Arabidopsis* seeds provide an ideal environment for proliferation of aberrant nuclei released from the normal controls of the mitotic cell cycle. Although research with yeast and *Drosophila* will continue to provide the most comprehensive picture of mitosis and cytokinesis in eukaryotes, further analysis of *ttn* mutants should help to establish the significance of these basic cellular processes

for plant development. In addition, *TTN* genes may have played an important role in the evolution of critical differences in mitosis and cell cycle control between the developing embryo and endosperm tissue of angiosperms.

Results

Identification of titan mutants

Over 500 mutants disrupted in embryogenesis have been isolated and characterized in *Arabidopsis* (Goldberg *et al.*, 1994; Jürgens *et al.*, 1994; Meinke, 1995). The 250 *emb* mutants in our collection were originally placed into different classes based on terminal phenotypes (Meinke, 1994). Target *EMB* genes have been mapped relative to visible markers (Franzmann *et al.*, 1995), but early defects in mutant seeds have generally remained uncharacterized.

We present here the results of a Nomarski-based screen of mutant seeds that was designed to document the types of cellular abnormalities present in our collection. Approximately 150 mutants were chosen for this study, most with embryos arrested before the cotyledon stage. Mutant seeds were removed from heterozygous siliques at four different stages, cleared in Hoyers solution, and examined under a light microscope. The extent of embryo and endosperm development was noted for each mutant by analyzing seeds at the linear and cotyledon stages of normal development. Early defects were identified by examining seeds from siliques at the globular and heart stages. About 100 seeds were examined for each mutant. Many interesting variations were found in morphogenesis and cell differentiation in the embryo, including alterations in cytokinesis, pattern formation, and suspensor development.

The most striking abnormality identified was the presence of giant endosperm nuclei in seeds from two pre-globular mutants (*emb127* and *emb133*) in which arrested embryos were not visible under a dissecting microscope. These mutants were subsequently named *ttn1* (*emb133*) and *ttn2* (*emb127*). Both of these mutants were generated in the Wassilewskija ecotype following *Agrobacterium*-mediated seed transformation (Castle *et al.*, 1993; Feldmann, 1991). The *ttn3* mutation was not identified based on embryo phenotype; mutant seeds were found segregating in another family (*emb293*) chosen for analysis. A monogenic *ttn3* line was then established by outcrossing this double mutant parent and scoring immature seeds from segregating subfamilies. All three *titan* mutants described in this report have enlarged endosperm nuclei but they differ in other aspects of seed development. The mutant embryo is composed of a few giant cells in *ttn1*, several small cells in *ttn2*, and in *ttn3* germinates to produce a normal plant. Other distinguishing features of these *ttn* mutants are summarized in Table 1.

Table 1. Comparison of seed phenotypes of *titan* mutants

Mutant seed phenotype	<i>titan1</i>	<i>titan2</i>	<i>titan3</i>
Endosperm phenotype:			
Nuclear size	Giant	Giant	Giant
Chromosome condensation	Present	Present	Present
Mitotic figures	Absent	Absent	Giant
Nuclear migration ^a	Absent	Present	Present
Cell wall formation ^b	Absent	Absent	Present
Embryo phenotype:			
Nuclear size	Giant	Normal	Normal
Cell morphology	Giant	Aborted	Normal
Embryo viability	Inviable	Inviable	Viable

^aIn wild-type seeds, some endosperm nuclei migrate to the chalazal end of the embryo sac early in development.

^bEndosperm cellularization in wild-type seeds begins around the heart stage of embryo development.

Genetic analysis of *titan* mutants

The *ttn1* and *ttn2* mutants are embryonic lethals in which 25% of the seeds produced by heterozygous plants abort prior to desiccation. Mutant embryos cannot be rescued in culture and mutant seeds fail to germinate at maturity. Map locations for *ttn1* and *ttn2* have already been published (Franzmann *et al.*, 1995) and complementation tests performed with other linked mutants. The *ttn1* locus maps to the bottom of chromosome 3 at 85 cM on the classical genetic map (available at <http://mutant.lse.okstate.edu/>); *ttn2* is located at the top of chromosome 1 (29 cM). The *ttn3* locus was not previously defined and was difficult to map because immature siliques from each F_2 plant had to be examined with Nomarski optics to identify mutant seeds. Results from crosses with DP23 (*ch1, er, gl1, cer2, tt3*), DP24 (*er, bp, ttg, yi*), and DP28/*hy2* (*dis1, clv2, er, tt5, hy2*) placed the *ttn3* locus at the bottom of chromosome 5, within 10 cM of the *yi* locus. The proper orientation of *ttn3* relative to *yi* remains to be determined. One allele of each *titan* mutant has been identified to date. The *ttn1* and *ttn2* mutants are not tagged with a functional T-DNA insert. The *ttn3* mutant is tagged with a single insert based on a 3:1 ratio of kanamycin-resistant to kanamycin-sensitive seedlings on plates and the absence of wild-type plants among 196 resistant seedlings transplanted to soil and scored for the mutation.

The *titan1* phenotype

The *ttn1* mutant exhibits striking defects in both embryo and endosperm development. Examples of mutant seeds and wild-type controls are shown in Figure 1. Immature mutant seeds are filled with a watery endosperm and have a glassy appearance not encountered with other embryo-defective mutants. Patches of green tissue visible through the transparent seed coat correspond to enlarged masses

of endosperm cytoplasm. One such cytoplasmic mass is seen in Figure 1(h). These distinctive features of seed development may facilitate the isolation of additional mutants with related phenotypes in the future. The mutant embryo stops dividing early in development and contains at most four cells in the embryo proper and suspensor combined. In approximately 30% of seeds examined, division of the zygote was blocked entirely and the embryo was composed of a single cell. Incomplete cell walls are occasionally found in arrested embryos, consistent with a general disruption of cytokinesis. Developmental arrest of the mutant embryo is accompanied by extensive cell and nuclear enlargement (Figure 1c,g). In extreme cases, individual cells may reach 180 μ m in diameter late in development, corresponding to more than a 1500-fold increase in cell volume.

Mutant endosperm nuclei also undergo striking changes in size and morphology during seed development. Nucleoli have been examined in the most detail because they are more prominent than nuclei when examined with Nomarski optics. Mutant endosperm nucleoli may reach 60 μ m in diameter late in development (Figure 1d). This is larger than a wild-type globular embryo composed of more than 50 cells (Figure 1a). Mutant nucleoli frequently exhibit an unusual ring structure that is visible in both cleared seeds (Figure 1d) and sectioned material (Figure 1h). Staining characteristics indicate that these rings are enlarged nucleoli and that surrounding pale-staining regions correspond to nuclei. DAPI staining of whole mounts and sections through mutant seeds confirmed that increased nuclear volume was accompanied by a dramatic rise in DNA content (data not shown). Endosperm nuclei were also disrupted in their normal pattern of migration to the chalazal end of the seed early in development. No chalazal endosperm tissue was detected in *ttn1* seeds examined in sectioned material. The endosperm tissue in wild-type seeds contains several hundred nuclei when cellularization begins at the heart stage of development. The average number of endosperm nuclei per mutant seed remained constant at 12 ± 4 from the globular to cotyledon stages. The increase in nuclear volume observed late in endosperm development cannot therefore result simply from fusion of existing nuclei. Whether nuclear fusion occurs to a limited extent early in development remains to be determined.

Mitotic divisions of *ttn1* endosperm nuclei appear to be blocked at prophase. Examples of condensed chromosomes present in mutant seeds are shown in Figure 2. The irregular shape of these chromosome clusters suggests that the nuclear membrane has degenerated. Mitotic figures beyond prophase were not found in stained sections or cleared seeds. Endosperm nuclei are therefore able to enter mitosis but fail to progress through subsequent stages of the cell cycle. A similar pattern of aborted mitosis

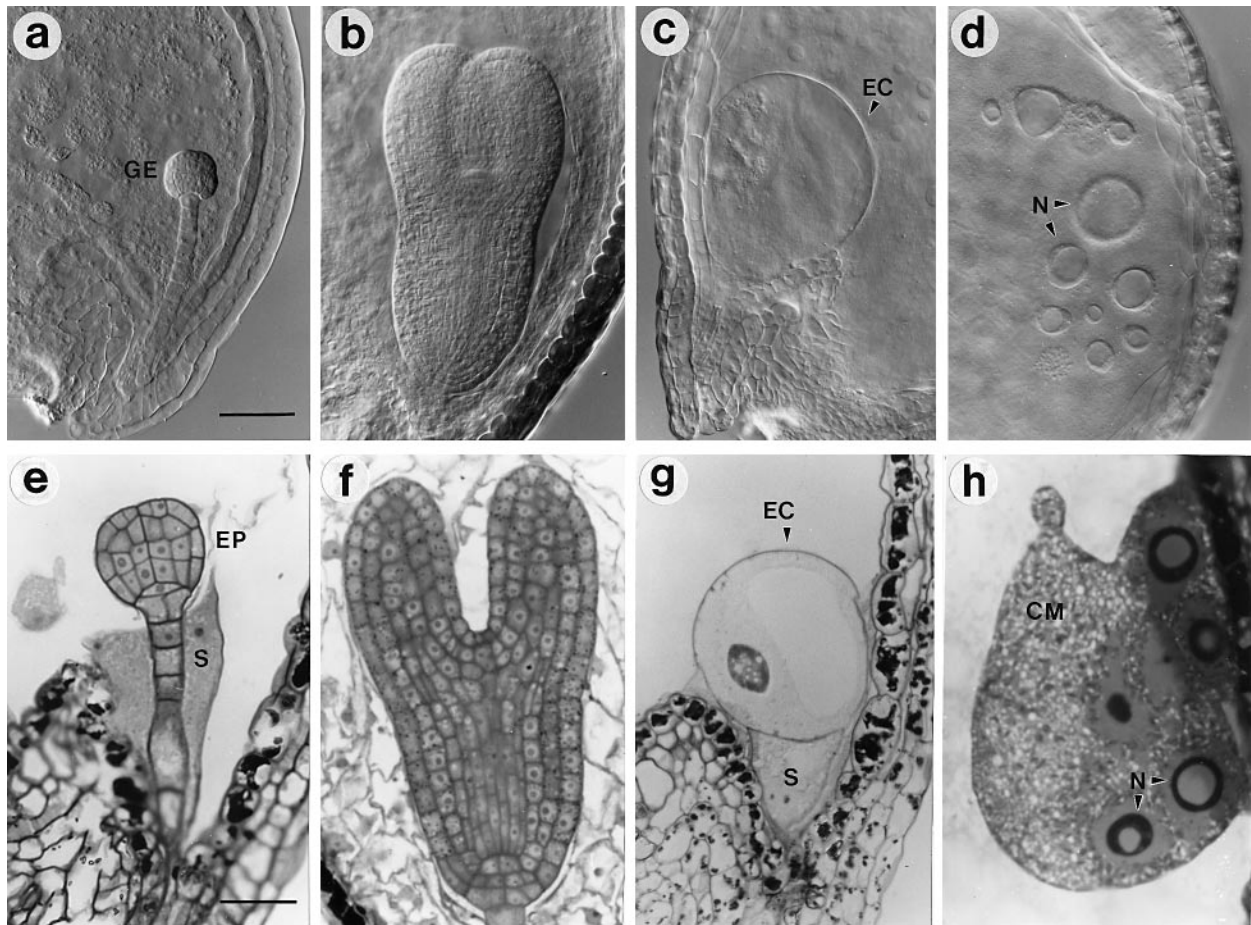


Figure 1. Dramatic enlargement of nuclei and embryo cells in *ttn1* seeds. (a–d) Nomarski optics of cleared wild-type seeds at the globular (a) and torpedo (b) stages of development and mutant seeds with enlarged embryo cell (c) and endosperm nuclei (d). All pictures were taken at the same magnification. Bar = 50 μ m. The mutant embryo cell (EC) and endosperm nucleoli (N) are larger than a wild-type globular embryo (GE). (e–h) Stained sections of wild-type (e, f) and mutant (g, h) seeds at stages close to those of cleared seeds pictured above. All pictures were taken at the same magnification. Bar = 25 μ m. Giant endosperm nucleoli (N) are visible within large cytoplasmic masses (CM). The mutant embryo cell (EC) and suspensor (S) are larger than a wild-type embryo proper (EP) at the globular stage.

was observed in the embryo. Mutant nuclei must later return to S phase in order to account for the mixture of giant nuclei and condensed chromosomes visible in mutant seeds. It therefore appears that repeated cycling between S phase, interphase and prophase is responsible for the nuclear enlargement found in *ttn1* seeds.

The *titan2* phenotype

There are several important differences between the *ttn1* and *ttn2* phenotypes. Both mutant heterozygotes produce seeds that are watery and glassy in appearance but *ttn2* seeds are often smaller and do not contain the prominent green spots found in *ttn1* seeds. The *ttn2* embryo is arrested early in development, with 1–2 cells in the embryo proper and 1–3 cells in the suspensor. Lethality does not occur before division of the zygote and is not accompanied by cell enlargement. A typical mutant embryo viewed with

Nomarski optics is shown in Figure 3(a). Cell division planes in the embryo proper and suspensor appear normal before developmental arrest. Cellular degeneration of the mutant embryo begins earlier in *ttn2* seeds than in *ttn1* seeds, often around the heart stage of normal development. Endosperm nuclei become enlarged, a feature common to all *titan* mutants, but the number of endosperm nuclei and the extent of nuclear enlargement in *ttn2* is somewhat reduced relative to *ttn1*. In addition, the average number of nucleoli is increased. An enlarged endosperm nucleus with several nucleoli of different sizes is shown in Figure 3(b). Nuclear migration does not appear to be disrupted because endosperm nuclei are present at the chalazal end of mutant seeds. Clusters of condensed chromosomes are visible throughout the endosperm tissue but more advanced mitotic figures are again missing. Some *ttn2* heterozygotes produce a low frequency of aborted seeds with enlarged endosperm nuclei associated with an

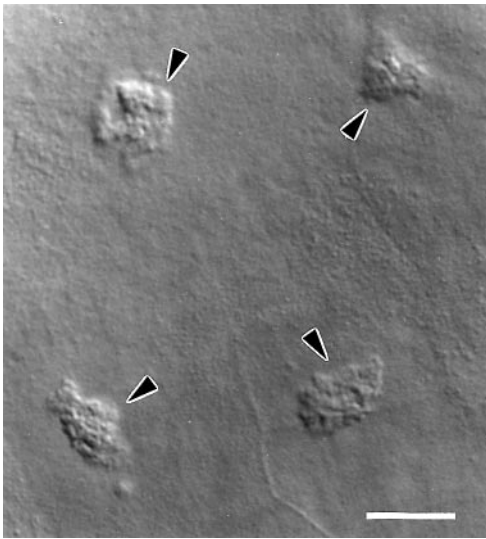


Figure 2. Chromosome morphology in *ttn1* seeds. Synchronous condensation of prophase chromosomes (arrowheads) from four endosperm nuclei within a single mutant seed. Bar = 30 μ m.

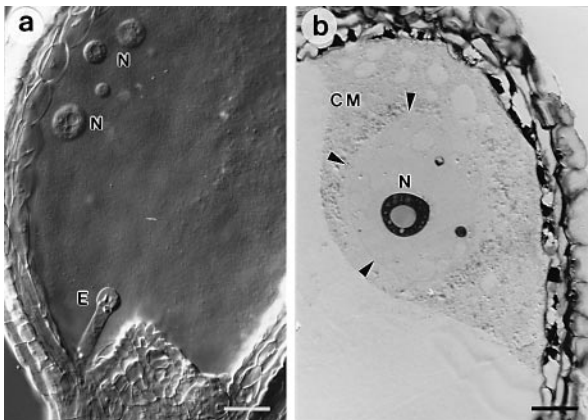


Figure 3. Embryonic arrest and enlargement of endosperm nuclei in *ttn2* seeds.

(a) Nomarski optics of a cleared mutant seed containing an embryo (E) arrested early in development without cell enlargement and several large endosperm nucleoli (N). Bar = 30 μ m.

(b) Section through part of a mutant seed containing an endosperm cytoplasmic mass (CM) and giant endosperm nucleus (margin defined by arrowheads) surrounding a dark-staining nucleolus (N). Bar = 30 μ m.

embryo arrested at a linear stage of development. The molecular basis of this partial suppression of the *ttn2* embryo phenotype remains to be determined.

The *titan3* phenotype

The primary effect of the *ttn3* mutation is on endosperm development. Enlarged endosperm nuclei with giant nucleoli are present at the globular and heart stages of embryo development. A cleared seed at this stage is shown in Figure 4(a). Migration of endosperm nuclei does not appear to be disrupted. In contrast to other *ttn* mutants, where the endosperm remains in a free nuclear state

throughout development, the *ttn3* endosperm begins to form cell walls at the appropriate time and then later in development becomes extensively cellularized. An immature seed with cellular endosperm is shown in Figure 4(b). The morphology of endosperm nuclei begins to change around the time of cellularization, with lobed and elongated nuclei becoming common. Multiple small nucleoli also become prominent at this stage of development.

Another unique feature of the *ttn3* phenotype is viability of the mutant embryo. Early stages of embryo development, as shown in Figure 4(a), are surprisingly normal considering the dramatic changes taking place in nuclear volume throughout the endosperm. Pattern formation in the early embryo is therefore not dependent upon maintenance of normal ploidy levels in the developing endosperm. Minor alterations in embryo morphology observed late in development, such as distorted cotyledons and twisted embryos, appear to result from changes in physical properties of the mutant endosperm. Mutant embryos survive desiccation and germinate to form a phenotypically normal plant, which produces 100% defective seeds with the expected phenotype following self-pollination. Another distinctive feature of the *ttn3* endosperm phenotype is the presence of giant mitotic figures with large numbers of chromosomes. As shown in Figure 5, enlarged mitotic figures ranging from prophase to telophase were readily seen in both cleared seeds and sectioned material. Abnormal spindles with multiple poles were occasionally observed. The number of chromosomes in a single mitotic figure was estimated in some cases to be several hundred. Mitotic figures were restricted to the free nuclear stage of endosperm development and were not found at later stages of endosperm cellularization.

Analysis of double mutants

Double mutants were constructed to study epistatic relationships and determine whether novel *titan* phenotypes could be created in different genetic backgrounds. Results of these studies are summarized in Table 2. Plants heterozygous for both mutations were identified by the presence of siliques containing mutant seeds with both parental phenotypes. These plants were expected to produce 1/16 double mutant seeds in addition to 3/16 mutant seeds of each parental class because the genes involved were not linked. Seeds homozygous recessive for both mutations were in some cases indistinguishable from one of the parental classes. Chi-square tests were then used to demonstrate that double mutant seeds were included among the more prevalent parental class. For example, 300 of the 533 mutant seeds produced by selfing of *ttn1/TTN1; ttn2/TTN2* double heterozygotes exhibited a typical *ttn2* phenotype. The remaining 233 mutant seeds exhibited a typical *ttn1* phenotype. No intermediate or novel phenotypes were

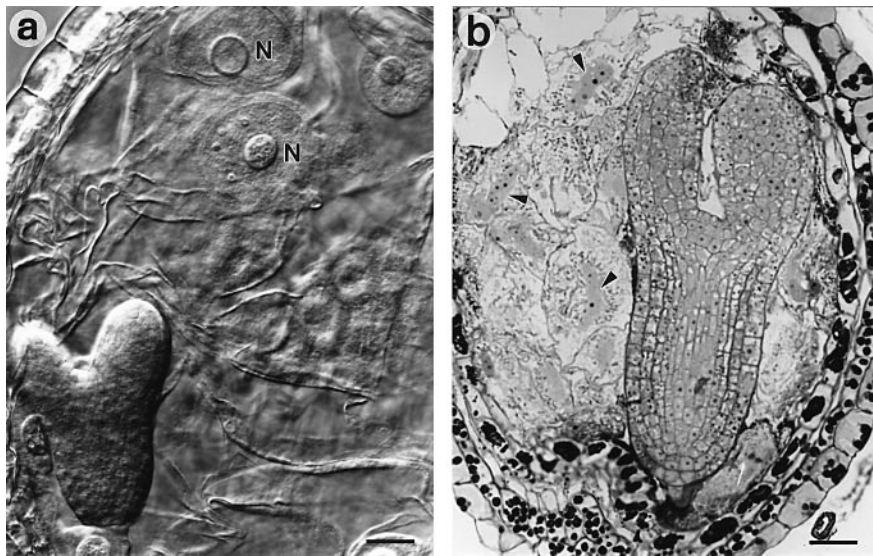


Figure 4. Normal embryo development and endosperm cellularization in *ttn3* seeds.

(a) Nomarski optics of a cleared mutant seed at the late heart stage of development. Several enlarged endosperm nucleoli (N) are visible. Bar = 30 μm.

(b) Section through a mutant seed at the torpedo stage of embryo development. Endosperm cellularization, elongation of endosperm nuclei (arrowheads), and diminution of endosperm nucleoli (small black dots) are evident at this stage. Bar = 30 μm.

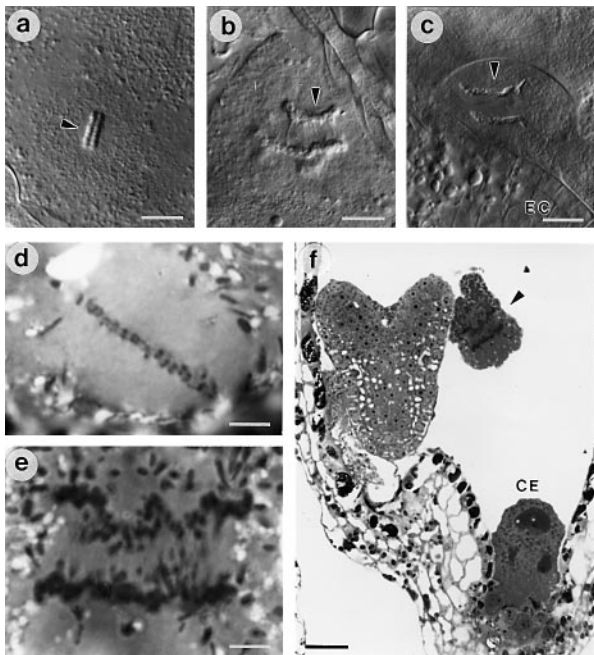


Figure 5. Giant mitotic figures in *ttn3* endosperm.

(a-c) Examples of metaphase, abnormal anaphase, and telophase stages (arrowheads) viewed with Nomarski optics. Embryo cells (EC) are visible in (c) for size reference. Bar = 15 μm.

(d) and (e) Stained sections of giant mitotic figures with large numbers of chromosomes. Bar = 5 μm.

(f) Section through a mutant seed containing a late heart-stage embryo and the giant endosperm mitotic figure (arrowhead) shown in (e). Chalazal endosperm tissue (CE) is also visible. Bar = 25 μm.

observed. These results are consistent with a 9:4:3 ratio of seed phenotypes where *ttn2* is epistatic to *ttn1* ($\chi^2 = 0.16$; not significant at $P = 0.05$) and inconsistent with a 9:3:3:1

Table 2. Summary of double mutant analysis^a

Parent		Parent	Phenotype of mutant seeds
<i>titan1</i>	x	<i>titan2</i>	<i>titan2</i>
<i>titan1</i>	x	<i>titan3</i>	<i>titan1</i>
<i>titan2</i>	x	<i>titan3</i>	<i>titan2</i>
<i>titan1</i>	x	<i>cyt1</i>	<i>titan1</i>
<i>titan2</i>	x	<i>cyt1</i>	<i>titan2</i>
<i>titan1</i>	x	<i>fass1</i>	<i>titan1</i>
<i>titan3</i>	x	<i>fass1</i>	Additive

^aCrosses were generally made between two heterozygous plants and putative double mutant seeds were identified as described in the text. Homozygous mutant plants were used for some *ttn3* crosses.

ratio where double mutants are lost during pollen development ($\chi^2 = 8.4$; significant at $P = 0.01$). A functional *TTN2* gene is therefore required for the dramatic cell enlargement observed in *ttn1* mutant seeds.

A similar approach was used to demonstrate that both *ttn1* and *ttn2* are epistatic to *ttn3*, and that introducing another mutation (*cyt1*) known to interfere with cytokinesis in the embryo does not enhance the severity of *ttn1* or *ttn2* phenotypes. Double mutants were also constructed with the *fass1* (*tonneau2*) mutation known to disrupt establishment of microtubular preprophase bands during embryo development. The *fs1* mutation did not alter the phenotype of *ttn1* seeds. Double mutant seeds exhibited a *ttn1* phenotype, which indicates that further disruption of microtubule organization does not alter the cell and nuclear enlargement characteristic of *ttn1*. The additive

effect observed with *fs1 ttn3* double mutants (*fs1* embryo phenotype; *ttn3* endosperm phenotype) was consistent with the restricted effects of each mutation on embryo and endosperm development. Seeds homozygous for *fs1* and *ttn2* were not analyzed because they could not be distinguished from the atypical embryos present in some *ttn2* parental lines.

Discussion

The *titan* (*ttn*) mutants described in this report define a novel class of *Arabidopsis* mutants disrupted in mitosis and cell cycle control during seed development. The aberrant cell cycles observed in these recessive mutants are among the most striking defects in mitosis described for a multi-cellular eukaryote. Based on the frequency of mutants obtained to date, we estimate that 2–3% of embryo-defective mutants exhibit a *titan* phenotype and that approximately 10–15 *TTN* genes are present in the *Arabidopsis* genome. Additional *ttn* mutants with related phenotypes have recently been found in our laboratory and by Wolfgang Lukowitz and Chris Somerville at the Carnegie Institute (Stanford, CA, USA). New alleles of *ttn1* and *ttn2* should become available once extensive mapping and allelism tests are completed. Duplicate *ttn3* alleles will be more difficult to identify because abnormalities are restricted to endosperm tissue. *TTN1* and *TTN2* appear to be required for development of both embryo and endosperm tissue. In contrast, *TTN3* may be endosperm-specific because mutant embryos develop into viable plants. Mutant seeds also differ in embryo and endosperm morphology. The *ttn* mutants therefore define a heterogeneous collection of genes with essential functions at different stages of the mitotic cell cycle in plants.

Two contrasting models for *TTN* gene function merit consideration. Based on mutant phenotypes and double mutant analysis, we conclude that *TTN* genes play a direct role in either cell cycle control or the structural mechanics of mitosis. An alternative model that *TTN* genes are required for the onset of cytokinesis can be discounted because endosperm defects in mutant seeds appear early in development, before the normal onset of cellularization. Several mutants blocked in endosperm cellularization have also been identified in our laboratory that do not exhibit a *ttn* phenotype. These results support the conclusion that enlargement of endosperm nuclei in *ttn* seeds does not result simply from a disruption of endosperm cellularization.

According to the first model of gene function, some *TTN* genes may correspond to known regulators of the eukaryotic cell cycle. A number of cell cycle genes have recently been identified in *Arabidopsis* (Ach *et al.*, 1997; Hirayama and Shinozaki, 1996; Shaul *et al.*, 1996; Xia *et al.*, 1996). The importance of some cell cycle regulators in

plant development has been explored through constitutive gene expression (Doerner *et al.*, 1996) and dominant negative mutations (Hemerly *et al.*, 1995). Many of these candidate genes can be discounted as targets for known *ttn* mutants because they map to different regions of the genome or have functions inconsistent with a *ttn* phenotype. The mutants described in this report are capable of initiating mitosis because some chromosome condensation has been observed. What appears to be missing is normal progression through later stages of mitosis. This may result from a disruption of checkpoint controls that monitor the completion of mitosis and prevent inappropriate DNA synthesis. Such checkpoint regulators represent promising candidates for *TTN* gene function.

An alternative model is that *TTN* genes help to establish the elaborate cytoskeletal network required for normal chromosome behavior during mitosis. Considerable progress has been reported in the identification of such genes from *Arabidopsis* (Asada and Collings, 1997; Huang *et al.*, 1996; Liu *et al.*, 1994). Disruption of chromosome condensation, chromatid separation, chromosome movement, or cytoskeletal organization could result in a *ttn* phenotype if replication continued in the absence of normal gene function. The absence of nuclear migration in *ttn1* endosperm tissue is potentially consistent with such a cytoskeletal defect. Some *ttn* mutants may therefore provide valuable insights into the genetic control of cytoskeletal organization in plants. Although the distribution of microtubules in young seeds and the effects of anti-microtubule drugs on cultured plant cells have been described previously (Liu *et al.*, 1995a; Webb and Gunning, 1991), the consequences of disrupting the cytoskeleton during seed development in *Arabidopsis* remain to be explored. Phenocopying *ttn* defects with chemical inhibitors of cytoskeletal organization could provide further support for the cytoskeletal model of *TTN* gene function.

A variety of mutants altered in nuclear morphology and chromosome behavior have been identified in other model organisms. For example, defects in microtubule organization and nuclear behavior during reproductive development have recently been described for the *lethal ovule2* and *indeterminate gametophyte* mutants of maize (Huang and Sheridan, 1996; Sheridan and Huang, 1997). Nuclear enlargement is much more limited than in *ttn* mutants but the cellular mechanisms involved may be related. Many additional mutants disrupted in mitosis and cell cycle control have been analyzed in *Drosophila*. Gatti and Baker (1989) screened larval lethals for mitotic abnormalities and found a variety of mutants with reduced mitotic indices, aberrations in chromosome condensation, and division failures that resulted in polyploid cells. Mutants with enlarged nuclei have also been identified among maternal-effect lethals of *Drosophila*. Three mutants (*plu*, *png*, *gnu*) have been analyzed in detail (Freeman *et al.*, 1986;

Shamanski and Orr-Weaver, 1991). Unfertilized mutant eggs contain a small number of giant nuclei derived from the haploid products of meiosis. DNA replication therefore occurs in the absence of either fertilization or mitosis. The *plu* gene encodes a small, ankyrin repeat protein that is present in oocytes and early embryos (Elfring *et al.*, 1997). The precise role of this protein in regulating entry into the S phase at fertilization remains to be determined. The activation of DNA synthesis without fertilization in *plu/png/gnu* mutants of *Drosophila* is reminiscent of inappropriate endosperm development found in *fie/fis* mutants of *Arabidopsis* (Chaudhury *et al.*, 1997; Ohad *et al.*, 1996). However, these endosperm mutants do not produce giant nuclei and the *titan* mutants do not exhibit defects in gametogenesis or fertilization. The relationship between *TTN* and *FIE/FIS* functions in plant development and similarities between these genes and cell cycle regulators in *Drosophila* remain to be determined.

The transition between diploidy and polyteny has also been analyzed in *Drosophila* (Sauer *et al.*, 1995; Smith and Orr-Weaver, 1991). Some genes function to maintain diploidy (Fuse *et al.*, 1994; Hayashi, 1996) while others block the inappropriate onset of mitosis in cells undergoing endoreduplication (Reed and Orr-Weaver, 1997). Polyploidy has been observed in several *Drosophila* mutants disrupted in cytokinesis (Karess *et al.*, 1991; Neufeld and Rubin, 1994; Ohkura *et al.*, 1997) and in numerous cell division mutants of *S. pombe* (Chang and Nurse, 1996; Simanis, 1995). Extensive genetic studies with *S. pombe* have allowed the molecular identification of large numbers of *cdc* genes with essential functions in mitosis and cytokinesis. Some of these genes encode proteins that influence assembly and placement of the contractile actin ring (Chang *et al.*, 1996). Others integrate mitosis with cytokinesis or function as checkpoint controls throughout the cell cycle (Heichman and Roberts, 1996; Woollard *et al.*, 1996). Extrapolating mutant phenotypes to higher plants, however, has often been difficult because the underlying mechanisms of cytokinesis are somewhat different. Analysis of mutants disrupted in mitosis and cell cycle control in other eukaryotes has therefore provided only limited clues to *TTN* gene identity.

One striking feature of the *titan* mutants identified to date is the dramatic variation observed in embryo cell morphology. The giant size of *ttn1* embryo cells contrasts with the small size of *ttn2* cells and the normal morphology of *ttn3* cells. Considerable attention has been given in recent years to elucidating the genetic control of pattern formation during plant embryo development (Jürgens *et al.*, 1994). Much less emphasis has been placed on understanding the genetic regulation of cell size during seed development. The extensive cell enlargement described here for *titan1* represents the

most striking change in cell architecture reported for an embryo-defective mutant of *Arabidopsis*. The developing seed with its nutritive endosperm tissue appears to provide an ideal environment for continued cell expansion. Some *ttn* mutants could therefore be used in the future to examine the regulation of cell size in relation to nuclear volume and basic metabolism during plant growth and development. The cell enlargement described here could also provide clues to the formation of giant feeding cells in *Arabidopsis* plants exposed to root-knot nematodes. Expression of cell cycle markers in these specialized cells has recently been analyzed in some detail (Niebel *et al.*, 1996).

Enlargement of the nucleolus is another intriguing feature of the *ttn* phenotype. The *ttn* mutants provide a unique opportunity to study basic questions of nucleolar organization in plants (Shaw *et al.*, 1996) and complement the extensive literature dealing with nucleolar structure in animal systems (Busch and Smetana, 1970). *Arabidopsis* has two nucleolus organizer regions (NORs) that have been analyzed at the molecular level (Copenhaver *et al.*, 1995; Haberer *et al.*, 1996). The presence of a single giant nucleolus in most *ttn1* endosperm nuclei suggests that replicated chromosomes remain organized with NORs associated in some manner following multiple rounds of DNA synthesis. This organization is not characteristic of all *ttn* phenotypes because additional nucleoli have been found in other mutants. Why giant nucleoli often assume a ring shape and what factors trigger the nucleolar reorganization observed late in *ttn3* endosperm development remain to be determined.

Some *TTN* genes may have played an important role in endosperm evolution by modulating differences in mitotic cell cycles during embryo and endosperm development. The origin of the endosperm tissue during plant evolution has been the subject of considerable interest in recent years (Friedman, 1994). The *ttn* mutants illustrate the dramatic effects that changes in single genes can have on endosperm morphology. We have previously shown that embryo-defective mutants can be used to study questions of cotyledon identity (Meinke, 1992; Meinke *et al.*, 1994) and suspensor cell identity (Schwartz *et al.*, 1994; Vernon and Meinke, 1994) during seed development. The *titan* phenotype described here may lead to a better understanding of endosperm identity and the molecular factors that enable the endosperm to progress through nuclear divisions distinct from the cell division cycles characteristic of the developing embryo. In order to address these questions at the molecular level, we are using TAIL-PCR to isolate the tagged *ttn3* allele, pursuing a map-based approach to clone the *TTN1* gene, and searching for additional *ttn* mutants among populations of insertional mutants defective in seed development.

Experimental procedures

Plant materials and growth conditions

The *titan* mutants described in this report were generated in the Wassilewskija ecotype following *Agrobacterium*-mediated seed transformation (Castle *et al.*, 1993; Feldmann, 1991). Mutant lines were maintained by growing plants in pots at $23 \pm 3^\circ\text{C}$ under 40 W cool white fluorescent lights kept on daily 16 h light/8 h dark cycles (Heath *et al.*, 1986). Heterozygous plants were identified by screening immature siliques for the presence of 25% defective seeds following self-pollination (Meinke, 1994). The *cyt1* mutant used for double mutant analysis was also isolated from the Feldmann collection. This mutant was originally known as *emb101* (Castle *et al.*, 1993) and is defective in cytokinesis during embryogenesis (Nickle and Meinke, 1998). The *fass1* mutant used for double mutant analysis was the *emb40* allele generated through EMS mutagenesis in the Columbia ecotype (Meinke, 1994). Mapping of *ttn3* relative to visible markers on the genetic map was performed as described by Franzmann *et al.* (1995), except that homozygous (*ttn3/ttn3*) plants were viable and could be identified in the F_2 generation by the presence of siliques with 100% rather than 25% defective seeds containing abnormal endosperm.

Microscopy of mutant seeds

Mutant seeds were removed from siliques at the globular, heart, linear and cotyledon stages of normal embryo development and cleared in Hoyers solution (7.5 g gum arabic, 100 g chloral hydrate, 5 ml glycerol in 30 ml water) as described by Meinke (1994). The fixation step was not essential and some preparations of Hoyers were diluted 20–30% with distilled water. Cleared seeds were examined using an Olympus BH-2 microscope equipped with Nomarski optics. Seeds prepared for light microscopy were removed intact from immature siliques, fixed overnight at room temperature in a solution containing 4% formaldehyde and 2.5% glutaraldehyde in 50 mM sodium cacodylate buffer at pH 7.4, postfixed with 1% OsO_4 in 100 mM cacodylate buffer for 1 h at room temperature, dehydrated in a graded ethanol series, infiltrated and embedded in LR White resin (Electron Microscopy Sciences, medium grade), and polymerized at 60°C for 16 h. Sections were cut at 1–2 μm with a glass knife on a rotary microtome (Reichert-Jung, Leica, Canada), stained with periodic acid-Schiff's reagent, and counter-stained with 1% amido black.

Acknowledgements

We thank Ken Feldmann for the invitation to screen transgenic plants for embryo-defective mutations; Linda Franzmann, Forrest Bath, Jennifer Goldman, Amy Davis, James Stanfield, and Jennifer Stanfield for assistance with screening and plant maintenance; and Todd Nickle for suggestions on light microscopy. The research was supported by grants from the National Science Foundation (Developmental Mechanisms Program) and S.R. Noble Foundation (Plant Biology Division) in Ardmore, Oklahoma.

References

Ach, R.A., Taranto, P. and Gruissem, W. (1997) A conserved family of WD-40 proteins binds to the retinoblastoma protein in both plants and animals. *Plant Cell*, **9**, 1595–1606.

- Asada, T. and Collings, D. (1997) Molecular motors in higher plants. *Trends Plant Sci.* **2**, 29–37.
- Assaad, F.F., Mayer, U., Wanner, G. and Jürgens, G. (1996) The *KEULE* gene is involved in cytokinesis in *Arabidopsis*. *Mol. Gen. Genet.* **253**, 267–277.
- Barton, N.R. and Goldstein, L.S.B. (1996) Going mobile: microtubule motors and chromosome segregation. *Proc. Natl Acad. Sci. USA*, **93**, 1735–1742.
- Busch, H. and Smetana, K. (1970) *The Nucleolus*. New York: Academic Press.
- Castle, L.A., Errampalli, D., Atherton, T.L., Franzmann, L.H., Yoon, E.S. and Meinke, D.W. (1993) Genetic and molecular characterization of embryonic mutants identified following seed transformation in *Arabidopsis*. *Mol. Gen. Genet.* **241**, 504–514.
- Chang, F. and Nurse, P. (1996) How fission yeast fission in the middle. *Cell*, **84**, 191–194.
- Chang, F., Woollard, A. and Nurse, P. (1996) Isolation and characterization of fission yeast mutants defective in the assembly and placement of the contractile actin ring. *J. Cell Sci.* **109**, 131–142.
- Chaudhury, A.M., Ming, L., Miller, C., Craig, S., Dennis, E.S. and Peacock, W.J. (1997) Fertilization-independent seed development in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA*, **94**, 4223–4228.
- Chen, Y.-C.S. and McCormick, S. (1996) *sidecar pollen*, an *Arabidopsis thaliana* male gametophytic mutant with aberrant cell divisions during pollen development. *Development*, **122**, 3243–3253.
- Christensen, C.A., King, E.J., Jordan, J.R. and Drews, G.N. (1997) Megagametogenesis in *Arabidopsis* wild-type and the *gf* mutant. *Sex. Plant Reprod.* **10**, 49–64.
- Copenhaver, G.P., Doelling, J.H., Gens, J.S. and Pikaard, C.S. (1995) Use of RFLPs larger than 100 kbp to map the position and internal organization of the nucleolus organizer region on chromosome 2 in *Arabidopsis thaliana*. *Plant J.* **7**, 273–286.
- Doerner, P., Jorgensen, J.-E., You, R., Steppuhn, J. and Lamb, C. (1996) Control of root growth and development by cyclin expression. *Nature*, **380**, 520–523.
- Edgar, B.A. and Lehner, C.F. (1996) Developmental control of cell cycle regulators: a fly's perspective. *Science*, **274**, 1646–1652.
- Efring, L.K., Axton, J.M., Fenger, D.D., Page, A.W., Carminati, J.L. and Orr-Weaver, T.L. (1997) *Drosophila* PLUTONIUM protein is a specialized cell cycle regulator required at the onset of embryogenesis. *Mol. Biol. Cell*, **8**, 583–593.
- Feldmann, K.A. (1991) T-DNA insertion mutagenesis in *Arabidopsis*: Mutational spectrum. *Plant J.* **1**, 71–82.
- Forsburg, S.L. and Nurse, P. (1991) Cell cycle regulation in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. *Annu. Rev. Cell Biol.* **7**, 227–256.
- Franzmann, L.H., Yoon, E.S. and Meinke, D.W. (1995) Saturating the genetic map of *Arabidopsis thaliana* with embryonic mutations. *Plant J.* **7**, 341–350.
- Freeman, M., Nüsslein-Volhard, C. and Glover, D.M. (1986) The dissociation of nuclear and centrosomal division in *gnu*, a mutation causing giant nuclei in *Drosophila*. *Cell*, **46**, 457–468.
- Friedman, W.E. (1994) The evolution of embryogeny in seed plants and the developmental origin and early history of endosperm. *Am. J. Bot.* **81**, 1468–1486.
- Fuse, N., Hirose, S. and Hayashi, S. (1994) Diploidy of *Drosophila* imaginal cells is maintained by a transcriptional repressor encoded by *escargot*. *Genes Devel.* **8**, 2270–2281.
- Gatti, M. and Baker, B.S. (1989) Genes controlling essential cell-cycle functions in *Drosophila melanogaster*. *Genes Devel.* **3**, 438–453.

- Goldberg, R.B., de Paiva, G. and Yadegari, R.** (1994) Plant embryogenesis: zygote to seed. *Science*, **266**, 605–614.
- Gorbasky, G.J.** (1995) Kinetochores, microtubules and the metaphase checkpoint. *Trends Cell Biol.* **5**, 143–148.
- Haberer, G., Fischer, T.C. and Torres-Ruiz, R.A.** (1996) Mapping of the nucleolus organizer region on chromosome 4 in *Arabidopsis thaliana*. *Mol. Gen. Genet.* **250**, 123–128.
- Hayashi, S.** (1996) A Cdc2 dependent checkpoint maintains diploidy in *Drosophila*. *Development*, **122**, 1051–1058.
- Heath, J.D., Weldon, R., Monnot, C. and Meinke, D.W.** (1986) Analysis of storage proteins in normal and aborted seeds from embryo-lethal mutants of *Arabidopsis thaliana*. *Planta*, **169**, 304–312.
- Heichman, K.A. and Roberts, J.M.** (1996) The yeast *CDC16* and *CDC27* genes restrict DNA replication to once per cell cycle. *Cell*, **85**, 39–48.
- Hemerly, A., de Almeida Engler, J., Bergounioux, C., Van Montagu, M., Engler, G., Inzé, D. and Ferreira, P.** (1995) Dominant negative mutants of the Cdc2 kinase uncouple cell division from iterative plant development. *EMBO J.* **14**, 3925–3936.
- Hirayama, T. and Shinozaki, K.** (1996) A *cdc5+* homolog of a higher plant, *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA*, **93**, 13371–13376.
- Huang, B.-Q. and Sheridan, W.F.** (1996) Embryo sac development in the maize *indeterminate gametophyte1* mutant: abnormal nuclear behavior and defective microtubule organization. *Plant Cell*, **8**, 1391–1407.
- Huang, S.R., McDowell, J.M., Weise, M.J. and Meagher, R.B.** (1996) The *Arabidopsis* profilin gene family: evidence for an ancient split between constitutive and pollen-specific profilin genes. *Plant Physiol.* **111**, 115–126.
- Hülkamp, M., Parekh, N.S., Grini, P., Schneitz, K., Zimmermann, I., Lolle, S.J. and Pruitt, R.E.** (1997) The *STUD* gene is required for male-specific cytokinesis after telophase II of meiosis in *Arabidopsis thaliana*. *Devel. Biol.* **187**, 114–124.
- Jacobs, T.W.** (1995) Cell cycle control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**, 317–339.
- Jürgens, G., Torres Ruiz, R.A. and Berleth, T.** (1994) Embryonic pattern formation in flowering plants. *Annu. Rev. Genet.* **28**, 351–371.
- Karess, R.E., Chang, X.-J., Edwards, K.A., Kulkarni, S., Aguilera, I. and Kiehart, D.P.** (1991) The regulatory light chain of nonmuscle myosin is encoded by *spaghetti-squash*, a gene required for cytokinesis in *Drosophila*. *Cell*, **65**, 1177–1189.
- King, R.W., Deshaies, R.J., Peters, J.-M. and Kirschner, M.W.** (1996) How proteolysis drives the cell cycle. *Science*, **274**, 1652–1659.
- Koshland, D. and Strunnikov, A.** (1996) Mitotic chromosome condensation. *Annu. Rev. Cell Devel. Biol.* **12**, 305–333.
- Liu, C.-M., Johnson, S. and Wang, T.L.** (1995b) *cyd*, a mutant of pea that alters embryo morphology is defective in cytokinesis. *Devel. Genet.* **16**, 321–331.
- Liu, B., Joshi, H.C. and Palevitz, B.A.** (1995a) Experimental manipulation of gamma-tubulin distribution in *Arabidopsis* using anti-microtubule drugs. *Cell Motil. Cytoskel.* **31**, 113–129.
- Liu, B., Joshi, H.C., Wilson, T.J., Silflow, C.D., Palevitz, B.A. and Snustad, D.P.** (1994) Gamma-tubulin in *Arabidopsis*: gene sequence, immunoblot, and immunofluorescence studies. *Plant Cell*, **6**, 303–314.
- Liu, Z., Running, M.P. and Meyerowitz, E.M.** (1997) *TSO1* functions in cell division during *Arabidopsis* flower development. *Development*, **124**, 665–672.
- Lukowitz, W., Mayer, U. and Jürgens, G.** (1996) Cytokinesis in the *Arabidopsis* embryo involves the syntaxin-related *KNOLLE* gene product. *Cell*, **84**, 61–71.
- Marc, J.** (1997) Microtubule-organizing centres in plants. *Trends Plant Sci.* **2**, 223–230.
- McClinton, R.S. and Sung, Z.R.** (1997) Organization of cortical microtubules at the plasma membrane in *Arabidopsis*. *Planta*, **201**, 252–260.
- Meinke, D.W.** (1992) A homoecotic mutant of *Arabidopsis thaliana* with leafy cotyledons. *Science*, **258**, 1647–1650.
- Meinke, D.W.** (1994) Seed development in *Arabidopsis thaliana*. In *Arabidopsis* (Meyerowitz, E.M. and Somerville, C.R., eds). New York: Cold Spring Harbor Laboratory Press, pp. 253–295.
- Meinke, D.W.** (1995) Molecular genetics of plant embryogenesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **46**, 369–394.
- Meinke, D.W., Franzmann, L.H., Nickle, T.C. and Yeung, E.C.** (1994) Leafy cotyledon mutants of *Arabidopsis*. *Plant Cell*, **6**, 1049–1064.
- Miyazaki, W.Y. and Orr-Weaver, T.L.** (1994) Sister-chromatid cohesion in mitosis and meiosis. *Annu. Rev. Genet.* **28**, 167–187.
- Nasmyth, K.** (1996a) Viewpoint: putting the cell cycle in order. *Science*, **274**, 1643–1645.
- Nasmyth, K.** (1996b) At the heart of the budding yeast cell cycle. *Trends Genet.* **12**, 405–412.
- Neufeld, T.P. and Rubin, G.M.** (1994) The *Drosophila peanut* gene is required for cytokinesis and encodes a protein similar to yeast putative bud neck filament proteins. *Cell*, **77**, 371–379.
- Nickle, T.C. and Meinke, D.W.** (1998) A cytokinesis-defective mutant of *Arabidopsis* (*cyt1*) characterized by embryonic lethality, incomplete cell walls, and excessive callose. *Plant J.* **15**, 321–332.
- Niebel, A., Engler, J.A., Hemerly, A., Ferreira, P., Inzé, D., Van Montagu, M. and Gheysen, G.** (1996) Induction of *cdc2a* and *cyt1At* expression in *Arabidopsis thaliana* during early phases of nematode-induced feeding cell formation. *Plant J.* **10**, 1037–1043.
- Nurse, P.** (1994) Ordering S phase and M phase in the cell cycle. *Cell*, **79**, 547–550.
- Ohad, N., Margossian, L., Hsu, Y.-C., Williams, C., Repetti, P. and Fischer, R.L.** (1996) A mutation that allows endosperm development without fertilization. *Proc. Natl Acad. Sci. USA*, **93**, 5319–5324.
- Ohkura, H., Torok, T., Tick, G., Hoheisel, J., Kiss, I. and Glover, D.M.** (1997) Mutation of a gene for a *Drosophila* kinesin-like protein, *Klp38B*, leads to failure of cytokinesis. *J. Cell Sci.* **110**, 945–954.
- Peirson, B.N., Bowling, S.E. and Makaroff, C.A.** (1997) A defect in synapsis causes male sterility in a T-DNA-tagged *Arabidopsis thaliana* mutant. *Plant J.* **11**, 659–669.
- Pines, J.** (1994) Protein kinases and cell cycle control. *Semin. Cell Biol.* **5**, 399–408.
- Reed, B.H. and Orr-Weaver, T.L.** (1997) The *Drosophila* gene *morula* inhibits mitotic functions in the endo cell cycle and the mitotic cell cycle. *Development*, **124**, 3543–3553.
- Sauer, K., Knoblich, J.A., Richardson, H. and Lehner, C.F.** (1995) Distinct modes of cyclin E/cdc2c kinase regulation and S-phase control in mitotic and endoreduplication cycles of *Drosophila* embryogenesis. *Genes Devel.* **9**, 1327–1339.
- Schwartz, B.W., Yeung, E.C. and Meinke, D.W.** (1994) Disruption of morphogenesis and transformation of the suspensor in abnormal suspensor mutants of *Arabidopsis*. *Development*, **120**, 3235–3245.
- Shamanski, F.L. and Orr-Weaver, T.L.** (1991) The *Drosophila plutonium* and *pan gu* genes regulate entry into S phase at fertilization. *Cell*, **66**, 1289–1300.
- Shaul, O., Van Montagu, M. and Inzé, D.** (1996) Regulation of cell division in *Arabidopsis*. *Crit. Rev. Plant Sci.* **15**, 97–112.
- Shaw, P.J., Beven, A.F., Wells, B., Highett, M.I. and Jordan, E.G.** (1996) The organization of nucleolar activity in plants. *J. Microsc.* **181**, 178–185.

- Sheridan, W.F. and Huang, B.-Q.** (1997) Nuclear behavior is defective in the maize (*Zea mays* L.) *lethal ovule2* female gametophyte. *Plant J.* **11**, 1029–1041.
- Simanis, V.** (1995) The control of septum formation and cytokinesis in fission yeast. *Semin. Cell Biol.* **6**, 79–87.
- Smith, A.V. and Orr-Weaver, T.L.** (1991) The regulation of the cell cycle during *Drosophila* embryogenesis: the transition to polyteny. *Development*, **112**, 997–1008.
- Spielman, M., Preuss, D., Li, F.-L., Browne, W.E., Scott, R.J. and Dickinson, H.G.** (1997) *TETRASPORE* is required for male meiotic cytokinesis in *Arabidopsis thaliana*. *Development*, **124**, 2645–2657.
- Staehein, L.A. and Hepler, P.K.** (1996) Cytokinesis in higher plants. *Cell*, **84**, 821–824.
- Staiger, C.J. and Cande, W.Z.** (1990) Microtubule distribution in *dv*, a maize meiotic mutant defective in the prophase to metaphase transition. *Devel. Biol.* **138**, 231–242.
- Staiger, C.J. and Cande, W.Z.** (1992) *Ameiotic*, a gene that controls meiotic chromosome and cytoskeletal behavior in maize. *Devel. Biol.* **154**, 226–230.
- Stern, B. and Nurse, P.** (1996) A quantitative model for the *cdc2* control of S phase and mitosis in fission yeast. *Trends Genet.* **12**, 345–350.
- Stillman, B.** (1996) Cell cycle control of DNA replication. *Science*, **274**, 1659–1664.
- Traas, J., Bellini, C., Nacry, P., Kronenberger, J., Bouchez, D. and Caboche, M.** (1995) Normal differentiation patterns in plants lacking microtubular preprophase bands. *Nature*, **375**, 676–677.
- Vernon, D.M. and Meinke, D.W.** (1994) Embryogenic transformation of the suspensor in *twin*, a polyembryonic mutant of *Arabidopsis*. *Devel. Biol.* **165**, 566–573.
- Webb, M.C. and Gunning, B.E.S.** (1991) The microtubular cytoskeleton during development of the zygote, proembryo and free-nuclear endosperm in *Arabidopsis thaliana* (L.) Heynh. *Planta*, **184**, 187–195.
- Woollard, A., Basi, G. and Nurse, P.** (1996) A novel S phase inhibitor in fission yeast. *EMBO J.* **15**, 4603–4612.
- Xia, G., Ramachandran, S., Hong, Y., Chan, Y.-S., Simanis, V. and Chua, N.-H.** (1996) Identification of plant cytoskeletal, cell cycle-related and polarity-related proteins using *Schizosaccharomyces pombe*. *Plant J.* **10**, 761–769.