Plant Cytokinesis. Exploring the Links

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Cytokinesis is the partitioning of the cytoplasm following nuclear division. This process presents a number of challenges for the plant cell: first, to avoid losing or bisecting the nucleus, this event needs to be carefully coordinated with respect to the nuclear cycle in space and in time. Second, a structure as complex as the plant cell wall needs to be laid down during the brief period of time between anaphase and telophase. The spatial and temporal regulation of cytokinesis requires a series of links between the nuclear cycle, the cell cortex, the Golgi apparatus, and the membrane trafficking apparatus. A number of genes have recently been identified that could enable us to probe such links.

Cytokinesis in higher plants may be considered as a specialized form of secretion. At the end of anaphase, Golgi-derived secretory vesicles carrying cell wall materials are transported to the equator of a dividing cell. Fusion of these vesicles gives rise to a membrane-bound compartment, the cell plate. The cell plate expands from the middle out (centrifugally) until it reaches the “zone of attachment” or division site on the mother cell wall. Once this attachment has taken place, the cell plate undergoes a complex process of maturation during which callose is replaced by cellulose and pectin (Samuels et al., 1995, and references therein).

Two cytoskeletal arrays, the preprophase band (PPB) and the phragmoplast, play central roles in cytokinesis in the somatic cells of higher plants. The PPB, a transient ring of cortical microtubules (MTs) and actin filaments, appears in late S phase, narrows throughout G2, and disappears during prophase when the nuclear envelope (NE) breaks down. The phragmoplast is an array of MTs and actin filaments present at the equator of a dividing cell during the anaphase to telophase transition (for review, see Assaad et al., 1997).

This review focuses on a small number of genes identified by mutation and whose molecular identities have recently been determined. These include TITAN5, TAN1, KEULE, KORRIGAN, and CYT1, required for cytokinesis in the somatic cells of higher plants (McElver et al., 2000; Zuo et al., 2000; Assaad et al., 2001; Lukowitz et al., 2001; Smith et al., 2001). In addition, reverse genetics has implicated the mitogen-activated protein (MAP) kinase kinase kinase NPK1 in cytokinesis (Nishihama et al., 2001). A number of important insights into plant cytokinesis have been gained from localization, drug, inhibitor, or antibody injection studies, reviewed elsewhere (Sylvester, 2000). Variant forms of plant cytokinesis specific to certain cell types are the subject of a recent review (Otegui and Staehelin, 2000).

Three questions are posed. First, how is cytokinesis regulated in space? Second, how is cytokinesis regulated in time? And third, what are the mechanisms underlying the execution of cytokinesis? The master choreographer of cytokinesis is the nucleus. Phragmoplast MTs are thought to be remnants of the mitotic spindle, which acts as a scaffold to ensure continuity in space and time between the nucleus and the cell equator. A number of processes underlying cytokinesis, including cytoskeletal reorganization, the biosynthesis and packaging of cell wall polymers, and vesicle traffic, need to be tightly regulated with respect to the nuclear cycle (see Fig. 1). It is possible that some of these processes are regulated by the numerous kinases found at the phragmoplast.

SPATIAL CONTROL OF CYTOKINESIS

A Spatial Cue Is Laid Down Early in the Cell Cycle and Marks the Division Site

Elegant experiments involving the displacement of nuclei or immature cell plates by centrifugation in moss protonemata or stamen hair cells have clearly demonstrated that the position of the interphase nucleus determines the division plane (Mineyuki and Gunning, 1990; Murata and Wada, 1991). Within a narrow window of time during the cell cycle, the nucleus is competent to dictate the position of the PPB that, in turn, marks the future division site (Murata and Wada, 1991). In stamen hair cells, immature cell plates displaced by centrifugation curve toward the site formerly occupied by the PPB (Mineyuki and Gunning, 1990). Thus, a spatial cue is laid down early during the cell cycle (Fig. 1A) and, later during the cell cycle, the nascent cell plate is guided toward this cue (Fig. 1D).

How Does the Interphase Nucleus Determine the Division Site?

A relay of links between the nucleus, the PPB, and the cell cortex/plasma membrane/cell wall may be
Figure 1. Plant cytokinesis: exploring the links. A. The division site is established during preprophase. The nuclear surface organizes the PPB (1), which may establish spatial cues (orange ovals; 2). B. Cell wall polymers such as xyloglucans are synthesized at the Golgi, packaged into vesicles, and transported to the equator of a dividing cell. C. The cell plate arises via fusion of Golgi-derived vesicles at the equator. The plate expands through the addition of secretory vesicles at the periphery, the deposition of callose in the lumena of the vesicular network, and via the concentric displacement of MTs toward the cell cortex (compare MTs in B, C, and D). Callose synthesis occurs within the cell plate, and this may be triggered by high concentrations of membrane-associated calcium. D. The phragmoplast is guided toward the spatial cue established during preprophase, MTs are now reoriented, with their minus ends at the equator and their plus ends toward the cell cortex. E. The cell plate fuses with the parental membranes and cell wall at the division site. Cellulose biosynthesis occurs at the plasma membrane; pectin biosynthesis takes place within the Golgi. F. The biosynthesis and/or packaging of cell wall polymers, membrane traffic, a MAP kinase cascade, and cytoskeletal organization are presumably coordinated with respect to cell cycle progression. How this relates to anaphase-promoting complex (APC) activation is unclear. Also, the targets of the MAP kinase remain to be determined. PPB and phragmoplast MTs are shown in red. Black arrows highlight established links and purple arrows potential links. Genes required for plant cytokinesis are shown in blue. The NE breaks down during mitosis and reassembles during telophase. The immature cell plate, rich in callose and also containing xyloglucans, is colored green, and the mature walls containing cellulose and pectin are colored in brown. Modeled according to Samuels et al. (1995) and Moore and Staehelin (1988); also see text.
implicated. First, the surfaces of plant nuclei have been shown to possess all the properties of MT organizing centers (MTOCs). These properties include: (a) the ability to nucleate and organize MTs, (b) the capacity to establish and/or anchor the minus ends of MTs, and (c) the presence at the nuclear surface of gamma tubulin and other proteins characteristic of animal MTOCs (Stoppin et al., 1994). Thus, it is likely that perinuclear MTOCs, which are associated with the NE, organize the PPB during late S-phase.

The division site plays an important role in cell wall maturation. Due to their high content in callose, immature cell plates are fluid and wrinkled. In contrast, mature cell plates are stiff and flat. This change occurs after the expanding cell plate has reached the division site and is accompanied by callose removal and cellulose and pectin deposition. In 1990, Mineyuki and Gunning proposed that the division site is established by: “(1) localized deposition of insertion and maturation factors in a latent form; and (2) provision of a means that, later on, will guide the leading edge of the centrifugally extending phragmoplast to the site. Once the new wall has attached, the factors are activated and utilized to insert, anchor and integrate new wall and contribute centripetally to its development... The PPB’s raison d’être is to... provide the necessary spatial guidance... for the localized deposition of the(se) factors.” Evidence for this model includes the following observations: (a) Cell plate maturation occurs if the nascent cell plate is inserted at the division site, but not if it is caused to insert elsewhere; (b) cell wall stubs develop from the outside in (centripetally) in cytokinesis-defective mutants or in caffeine-treated cells (see Fig. 2C below); (c) visible alterations of the parental cell wall occur at the site underlying the PPB; (d) PPBs do not occur in cells types in which the new walls are not inserted into parental walls; and (e) the position of the PPB and of the division site invariably correlate (see Mineyuki and Gunning, 1990, and references therein).

Genes Required for Cell Wall Orientation

The above model is strengthened by the analysis of plant mutants impaired in their ability to orient cell walls. The Arabidopsis fas and tonneau mutants, as well as the tangled 1 mutants of maize (Zea mays), are characterized by misoriented cell walls, especially during asymmetric or longitudinal divisions; whereas fas and tonneau mutants altogether lack PPBs, these rings of cortical MTs are often misoriented in tangled mutants (for review, see Nacry et al., 2000). In tonneau mutants, cortical MTs in general, including the PPB, are perturbed, yet the spindle and phragmoplast appear normal (Traas et al., 1995). It is possible that the FAS/TONNEAU gene products function at the nuclear surface to organize cortical MT arrays (Fig. 1A).

The TANGLED1 gene has been cloned and characterized (Smith et al., 2001). The gene encodes a highly basic protein bearing little sequence similarity to other proteins, yet possessing domains weakly homologous to the MT binding domain of vertebrate APC. TAN1 binds to MTs in vitro, possibly in a cell cycle-dependent manner, and proteins recognized by anti-TAN1 antibodies localize to the PPB, spindle, and phragmoplast in dividing cells, providing evidence that TANGLED1 may encode an MT-binding protein. It is interesting that in tangled mutants the leading edges of phragmoplasts are not guided to sites formerly occupied by PPBs. Mutant cell plates consistently do not undergo the flattening that accompanies cell plate maturation, but remain wrinkled (Smith et al., 2001, and references therein). In addition to orienting the PPB, TANGLED may be

Figure 2. Cell wall stubs and multinucleate cells in cytokinesis-defective embryos. A and B, Light micrographs of dermatogen stage embryos. A, keule mutant. B, Wild type. C, Electron micrograph of keule mutant embryo at dermatogen stage showing a non-vacuolated binucleate cell. The centripetal cell wall stub supports the model that the division site consolidates the immature cell plate (see text). N, Nucleus; P, protoderm. A star marks the uppermost cell of the suspensor. Arrows point to cell wall stubs and arrowheads to multinucleate cells. Reproduced, with permission, from Assaad et al. (1996; Figs. 4E, 4F, and 6E, © Springer-Verlag, Berlin). Bar = 1 μm in C.
implicated in the establishment of the division site during preprophase and/or may guide the leading edges of the phragmoplast to this site during cytokinesis (Fig. 1, A and D). The molecular identity of the spatial cue that determines the division site, as well as its localization to the cortex, plasma membrane, and/or cell wall, remain to be determined.

TEMPORAL REGULATION OF CYTOKINESIS

Cell Cycle Progression: Exiting Mitosis and Initiating Cytokinesis

The onset of cytokinesis is concomitant with exit from mitosis. In plants, progression through mitosis relies on the activity of the cyclinB-cdc2 complex (M-CDK) active during M phase. Shortly before anaphase, M-CDKs are thought to activate the APC, a ubiquitin ligase that in turn destroys the M-CDKs. Thus, cyclin-dependent kinases and the APC regulate each other to ensure timely progression through mitosis (Meijer and Murray, 2001; Nigg, 2001). A number of studies in budding and fission yeast (Saccharomyces cerevisiae and Saccharomyces pombe) support the notion that mitotic exit does not alone suffice for the initiation of cytokinesis. In fact, an additional kinase cascade triggers a cytokinetic pathway (Nigg, 2001). In budding yeast, the polo kinase CDC5 participates in APC activation and, in addition, appears to regulate the cytokinetic pathway by interacting with septins (Song and Lee, 2001). Because plants lack septins, it is not clear how these findings in yeast relate to plant cytokinesis.

A large number of kinases have been found at the phragmoplast (for list, see Nacry et al., 2000) and these are good candidates for orchestrating the onset and execution of cytokinesis. Compelling evidence that a MAP kinase cascade is required for plant cytokinesis comes from a recent study showing that kinase negative mutations in NPK1, a MAP kinase kinase kinase, disrupt cytokinesis in tobacco (Nicotiana tabacum) cells (Nishihama et al., 2001). NPK1 activity is up-regulated during late M phase. The protein is present in the nucleus during interphase, and at the equatorial zone of the phragmoplast where it may be required for phragmoplast expansion toward the cell cortex (Nishihama et al., 2001). Thus, NPK1 may provide continuity in space and in time between the interphase nucleus and the cell equator. A MAP kinase has been detected at the phragmoplast in alfalfa (Medicago sativa) cells (Bögøre et al., 1999) and its tobacco orthologue might be a target of NPK1. Two important questions remain unanswered: First, what signals activate NPK1? And, second, what are the targets of the putative MAP kinase cascade downstream of NPK1?

Cytokinesis and the Nuclear Cycle: One-Way Communication?

The multinucleate phenotype of cytokinesis-defective mutants suggest that nuclear division can be initiated and completed even if cytokinesis is incomplete. In contrast, cell wall stubs in such mutants are only observed in multinucleate cells, which suggests that cytokinesis can only be initiated once the nuclear cycle is complete. titan and pilz mutants, characterized by giant nuclei, consistently show marked cytokinesis defects (Liu and Meinke, 1998; for review, see Nacry et al., 2000). A simple hypothesis is that the cytokinesis defects observed in titan and pilz mutants are an indirect consequence of cell cycle arrest due to a primary defect in nuclear division. This is supported by the observation, based on tubulin stains, that not only cytokinesis but cell cycle progression in general is affected in pilz mutants (for review, see Nacry et al., 2000). A number of conserved cell cycle checkpoints known to monitor nuclear division and spindle assembly or orientation readily account for these observations (Nigg, 2001). In this context, it is interesting to note that cytokinesis defective mutants such as keule have enlarged nuclei (Assaad et al., 1996), suggesting that incomplete cytokinesis may also impact the nuclear cycle, though not in an absolute way. In general, however, the nucleus appears to play a dominant role in dictating the onset of cytokinesis.

PLANT CYTOKINESIS AS A SPECIALIZED FORM OF SECRETION

Role of the Golgi Apparatus in Cytokinesis

Drug studies have highlighted the importance of the Golgi apparatus in cytokinesis. The biosynthesis and assembly of numerous cell wall polysaccharides take place in the Golgi (Moore and Staehelin, 1988). In contrast, callose is synthesized within the cell plate and cellulose microfibrils are synthesized by cellulose synthases embedded in the plasma membrane, which explains why cell plate flattening and maturation only occur after fusion with the parental membrane and wall (Samuels et al., 1995, and references therein). Immature cell plates are rich in xyloglucans and devoid of pectins, whereas mature cross walls are rich in pectins and have low xyloglucan content (Moore and Staehelin, 1988; His et al., 2001). Because pectins and xyloglucans are synthesized in the Golgi and targeted to the cell plate, there appears to be a tight cell cycle regulation of Golgi activity and secretion (Moore and Staehelin, 1988; see Fig. 1).

The Membrane Dynamics of Cytokinesis

At the end of anaphase, Golgi-derived vesicles are transported toward the equator of a dividing cell. Vesicle fusion gives rise to the cell plate, assembled
within the phragmoplast. These vesicle trafficking events can be broken down into four steps: vesicle formation, transport, tethering/docking, and fusion (see Fig. 3A). As shown in Figure 3, each of these steps is highly regulated.

**Vesicle Formation**

An interesting recent finding is that one of the TITAN genes, TITAN5, encodes an ARF GTPase that may be implicated in vesicle formation during cell division (McElver et al., 2000, and references therein). This ARF may, for example, be required for NE breakdown into vesicles, and this could explain the giant nuclei observed in titan5 mutant embryos and endosperm. In addition to a role in regulating nuclear division, TITAN5 could also be required for vesicle formation during cytokinesis (McElver et al., 2000). With 45 members, ARFs represent a large gene family in Arabidopsis (www.Arabidopsis.org/Blast), and therefore it is likely that individual ARFs have specialized as opposed to multiple functions. Because plants lack orthologues of nuclear lamins (www.Arabidopsis.org/Blast), the identification of TITAN 5 provides a highly important and novel handle on the poorly understood process of NE dynamics and function.

**Vesicle Transport**

At anaphase, vesicles are transported to the equator of a dividing cell. Because the plus ends of phragmoplast MTs overlap at the equator, a plus end-directed motor such as kinesin would transport Golgi-derived vesicles to the equatorial region during cytokinesis. In addition, a minus end-directed motor such as myosin may play a role in vesicle translocation to the phragmoplast. Both kinesin and myosin have been found at the phragmoplast (Sylvestér, 2000).

**Vesicle Tethering or Docking**

Prior to fusion, vesicles are found docked at their target membranes. Docking is mediated by a number of protein interactions that connect Rab GTPases on vesicles with syntaxins on target membranes (Fig. 3B; Zerial and McBride, 2001). Although Arabidopsis contains a large family of Rab proteins, none has been implicated thus far in cytokinesis.

**Vesicle Fusion**

Vesicle fusion requires a specific “lock and key” interaction between syntaxins on target membranes and VAMPs or v-SNAREs on vesicle membranes. Members of the Sec1 superfamily of proteins appear capable of inducing conformational changes in syntaxins, “opening” or priming these for interactions with other SNAREs (Fig. 3B; for review, see Chen and Scheller, 2001). Molecular genetic analyses have identified two genes whose products, KEULE and KNOLLE,concertedly mediate membrane fusion events during cytokinesis. At a cellular level, knolle and keule mutants are characterized by multinucleate cells with gapped or incomplete cross walls (see Fig. 2; Assaad et al., 1996; Lukowitz et al., 1996). KNOLLE encodes a cytokinesis-specific syntaxin expressed in punctate, vesicle-like structures during M phase, and at the phragmoplast in dividing cells (Lukowitz et al., 1996; Lauber et al., 1997). KEULE encodes a Sec1 protein that has been shown to bind KNOLLE in vitro-binding assays (Assaad et al., 2001). The observation that vesicles accumulate but do not fuse at the equator of dividing cells in keule and knolle embryos (Waizenegger et al., 2000) indicates that, like the syntaxin KNOLLE, KEULE is required for vesicle fusion. The synthetic lethality of knolle keule double mutants has provided evidence that the two genes interact in vivo. Whereas cytokinesis is impaired but not blocked in keule and knolle mutants, which survive till the seedling stage, it is completely abolished in knolle keule double mutants that die as large, single-celled multinucleate embryos (Waizenegger et al., 2000).

What is the function of the KEULE/KNOLLE complex? Sec1 proteins are large and capable of multiple interactions, and thereby may integrate multiple signals. In yeast and animal cells, Sec1s couple the membrane fusion machinery on target membranes with the Rab GTP cycle on vesicle membranes (for review, see Zerial and McBride, 2001). In a similar manner, Sec1 proteins constitute a key link between exocytosis and the functional asymmetry and development of neural synapses (Butz et al., 1998). By analogy, an intriguing possibility is that KEULE may integrate cell cycle signals and transduce them to the cytokinetic vesicle fusion machinery by virtue of an interaction with the syntaxin KNOLLE. To this effect, a MAP kinase has been found to regulate the activity of a Rab/GDI complex in animal cells, thereby regulating endocytosis (Cavalli et al., 2001). Furthermore, phosphorylation by CDK5 regulates the nSec1-syntxin1 interaction in neural cells (Fletcher et al., 1999). Thus, the vesicle trafficking machinery could, for instance, be a target of the numerous Ser/Thr kinases (see Nacry et al., 2000), including MAP kinases, present at the phragmoplast.

**A Novel Membrane Compartment at the Plane of Division?**

The initial rounds of fusion at the cell equator most likely can be considered as “homotypic” in that they occur between like membranes, that is to say, between Golgi-derived vesicles. The new membrane compartment caused by vesicle fusion undergoes a series of visible alterations, including the appearance
of a membrane coat and clathrin-coated pits. Although these changes could formally be accompanied by a change in the identity of the cell plate membranes, they are most simply explained as reflecting membrane recycling from the cell plate. In contrast, fusion of the cell plate with the parental wall requires a heterotypic fusion between cell plate membranes and the plasma membrane. After this
event, the membranes that delineate the cell plate are contiguous with the plasma membrane, yet of Golgi-derived origin. In fact, during surface expansion and cell elongation, new endomembranes are also added to the plasma membrane.

As of when does the novel membrane compartment formed at the cell equator acquire a plasma membrane identity, and what is its original identity? Phylogenetic analysis places the syntaxin KNOLLE in a novel class of plant-specific syntaxins, as close to plasma membrane syntaxins as it is to endoplasmic reticulum/Golgi syntaxins, and in the same family as AtSYR1, which has been localized to the plasma membrane; KEULE’s closest homologs are animal Sec1s required for exocytosis (Lukowitz et al., 1996; Sanderfoot et al., 2000; www.msu.edu/approximately sanderfo/atsnare.htm). Green fluorescent protein fusions of the endo-1,4-β-glucanase KORRIGAN are targeted to both the plasma membrane and cell plate (Zuo et al., 2000). Evidence that the cell plate membranes in fact differ from the plasma membrane is provided by the observation that the plasma membrane H+-ATPase is excluded from the cell plate (Lauber et al., 1997). These observations support the view that the cell plate is a novel and distinct membrane compartment arising from a modified form of endomembrane,

Cell Plate Maturation

Cell plate maturation requires callose removal as well as cellulose and pectin deposition. Two genes that influence cell wall assembly, CYT1 and KORRIGAN, appear to be required for cell plate consolidation and maturation. Compared with the cell wall stubs observed as of the first zygotic divisions in keule and knolle mutants, the cell wall stubs observed in cyt1 and korrigan mutants first appear later in development and have only been documented in vacuolated cells (Assaad et al., 1996; Lukowitz et al., 1996; Nickle and Meinke, 1998; Zuo et al., 2000). This raises the question as to whether these cell wall stubs arise during cell expansion rather than cell division. It is, nonetheless, clear that the mechanical strength of cell plates is compromised in both mutant backgrounds. CYT1 encodes a Man-1-phosphate guanylyltransferase, required for N-glycosylation. The 5-fold reduction in cellulose content observed in cyt1 mutants readily accounts for its extreme cell wall defects, which include a high callose content and a diffuse distribution of unesterified pectins (Nickle and Meinke, 1998; Lukowitz et al., 2000). Noteworthy implications of these findings are that N-linked glycosylation appears to be required for cellulose biosynthesis, and that other polysaccharides such as callose and pectins may compensate for reduced cellulose levels. Reduced cellulose and altered pectin content have also been observed in korrigan mutants (His et al., 2001). As an endo-1,4-β-glucanase, KOR-
NS et al. (1999) A MAP kinase is activated late in plant mitosis and becomes localized to the plane of cell division. Plant Cell 11: 101–113