

# Redistribution of Golgi Stacks and Other Organelles during Mitosis and Cytokinesis in Plant Cells<sup>1[w]</sup>

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We have followed the redistribution of Golgi stacks during mitosis and cytokinesis in living tobacco BY-2 suspension culture cells by means of a green fluorescent protein-tagged soybean  $\alpha$ -1,2 mannosidase, and correlated the findings to cytoskeletal rearrangements and to the redistribution of endoplasmic reticulum, mitochondria, and plastids. In preparation for cell division, when the general streaming of Golgi stacks stops, about one-third of the peripheral Golgi stacks redistributes to the perinuclear cytoplasm, the phragmosome, thereby reversing the ratio of interior to cortical Golgi from 2:3 to 3:2. During metaphase, approximately 20% of all Golgi stacks aggregate in the immediate vicinity of the mitotic spindle and a similar number becomes concentrated in an equatorial region under the plasma membrane. This latter localization, the "Golgi belt," accurately predicts the future site of cell division, and thus forms a novel marker for this region after the disassembly of the preprophase band. During telophase and cytokinesis, many Golgi stacks redistribute around the phragmoplast where the cell plate is formed. At the end of cytokinesis, the daughter cells have very similar Golgi stack densities. The sites of preferential Golgi stack localization are specific for this organelle and largely exclude mitochondria and plastids, although some mitochondria can approach the phragmoplast. This segregation of organelles is first observed in metaphase and persists until completion of cytokinesis. Maintenance of the distinct localizations does not depend on intact actin filaments or microtubules, although the mitotic spindle appears to play a major role in organizing the organelle distribution patterns. The redistribution of Golgi stacks during mitosis and cytokinesis is consistent with the hypothesis that Golgi stacks are repositioned to ensure equal partitioning between daughter cells as well as rapid cell plate assembly.

Cell division requires the duplication of all critical cell components, their distribution into different domains within the cytoplasm, and the separation of these domains into two independent daughter cells. These events have been characterized most thoroughly for the genetic material, where duplication occurs during S phase of the cell cycle, followed by distribution of the resulting chromatids in mitosis, and the subsequent formation of two daughter cells by cytokinesis. The membranous organelles of eukaryotic cells can be expected to follow a similar sequence of events, the specific manifestation of which should be determined by the structural organization of these organelles and their functional requirements. In recent years the inheritance of the Golgi apparatus in cultured mammalian cells has received considerable interest (Lowe et al., 1998; Roth, 1999). In this case the single perinuclear Golgi complex breaks down at the onset of mitosis and reforms after cytokinesis. The distribution of Golgi membranes over a larger area of the cell is expected to aid in the even distribution of this organelle into the daughter cells (Warren and Wickner, 1996). Although the mechanics of this partitioning are still debated (Shima et al., 1998; Roth, 1999; Zaal et al.,

1999), there is general agreement that during this stage of the cell cycle, protein export from the endoplasmic reticulum (ER) is blocked and the secretory system is shut down (Warren, 1993; Farmaki et al., 1999). In contrast to the centralized Golgi of mammalian cells, the Golgi apparatus of plants cells consists of many independent stacks that continue to produce secretory products during all steps of mitosis and cytokinesis (Andreeva et al., 1998; Dupree and Sherrier, 1998). Thus the mode of Golgi partitioning in plant cells can be expected to differ from that in mammalian cells, reflecting both the different structural properties and the different functional requirements of plant cells.

Cytokinesis in terrestrial plants occurs by cell plate formation. This process entails the delivery of Golgi-derived vesicles carrying cell wall components to the plane of cell division and the subsequent fusion of these vesicles within this plane (Staehelin and Hepler, 1996; Heese et al., 1998). After formation of an early tubulo-vesicular network at the center of the cell, the initially labile cell plate consolidates into a tubular network and eventually a fenestrated sheet (Samuels et al., 1995). The cell plate grows outward from the center of the cell to the parental plasma membrane with which it will fuse, thus completing cell division. Formation and growth of the cell plate is dependent upon the phragmoplast, which is required for proper targeting of Golgi-derived vesicles to the cell plate. The phragmoplast is a complex assembly of microtubules (MTs), microfilaments

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(MFs), and ER elements, that assemble in two opposing sets perpendicular to the plane of the future cell plate during ana- and telophase (Staehelein and Hepler, 1996). As the cell plate matures in the central part of the cell, the phragmoplast disassembles in this region and new elements are added on its outside. This process leads to a steady expansion of the phragmoplast, and concomitantly, to a continuous retargeting of Golgi-derived vesicles to the growing edge of the cell plate. Once the cell plate reaches and fuses with the plasma membrane the phragmoplast disappears. This event not only marks the separation of the two daughter cells, but also initiates a range of biochemical modifications that transform the callose-rich, flexible cell plate into a cellulose-rich, stiff primary cell wall. In highly vacuolated cells these common mechanisms of cytokinesis are preceded by two additional events. First, the nucleus migrates to a central position in the cell defining the future division site. Subsequently, a cytoplasmic bridge, the phragmosome, is formed at this position in which mitosis and cytokinesis will occur (Lloyd, 1991).

The heavy dependence of cell plate formation on active Golgi stacks explains why plant cells, unlike mammalian cells, do not disassemble their secretion machinery during cell division. This dependence also raises the question as to whether there exists a special spatial relationship between the Golgi apparatus and the cytokinetic machinery. It has long been known that Golgi stacks appear in the vicinity of the phragmoplast. In fact this spatial proximity, together with the similarity in Golgi vesicle and cell plate staining, was the first indication that Golgi products may be involved in cell plate formation (Whaley and Mollenhauer, 1963). However, unlike the distribution of ER during mitosis and cytokinesis (Hepler, 1980, 1982), the relationship of Golgi stacks to the phragmoplast has received little attention. Only recently has a study attempted a rigorous assessment of Golgi stack distribution during cytokinesis. Using a double-staining approach, it was found that Golgi stacks approach the maturing cell plate more closely than mitochondria or plastids (Kawazu et al., 1995). But, since organelles were detected in chemically fixed and sectioned material, it was impossible to follow dynamic changes in living cells, or to visualize the three-dimensional distribution in an entire cell. Few studies have addressed the distribution of other organelles during cell division in terrestrial plants (Schopfer and Hepler, 1991), although proper segregation of organelles clearly is important for cell viability.

We have previously described the creation of an *in vivo* Golgi marker by expression of a fusion between a resident cis-Golgi protein (soybean  $\alpha$ -1,2 mannosidase [GmMan1]) and green fluorescent protein (GFP) in tobacco BY-2 suspension culture cells (Nebenführ et al., 1999). Using this tool we demonstrated that Golgi stacks can display characteristic translational

movement (cytoplasmic streaming) during interphase, which occurs along actin filaments. This stop-and-go movement is highly specific for individual stacks and presumably tightly regulated. The apparent regulation of Golgi stack movement suggests that their distribution in plant cells is not random, and that Golgi stacks can be recruited to specific locations where they perform secretory functions (Nebenführ et al., 1999). Cell plate formation is one example where Golgi products are needed at a specific location and may therefore serve as model for the postulated recruitment of Golgi stacks.

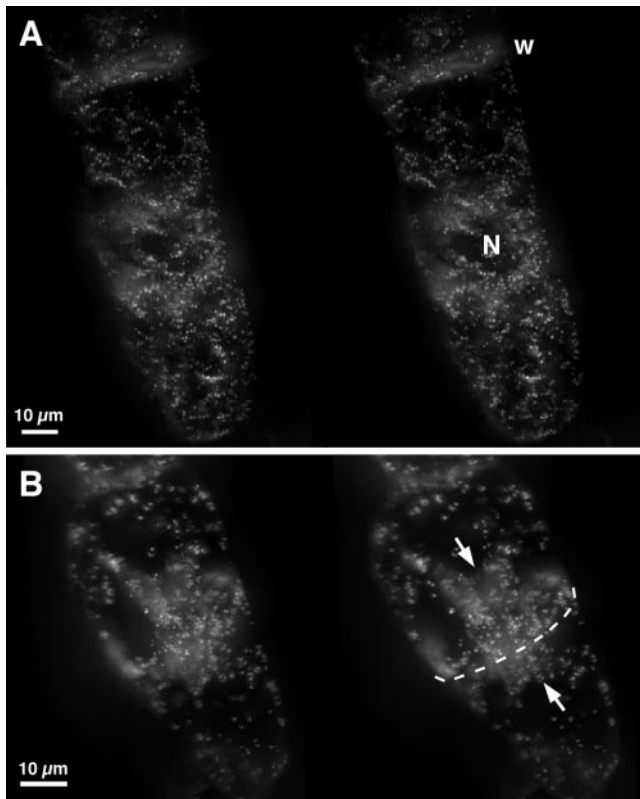
In this study we use the Golgi-localized fluorescent tag to quantitatively describe the three-dimensional distribution of Golgi stacks in living BY-2 cells during mitosis and cytokinesis. In addition we use an ER-targeted GFP construct and the fluorescent MitoTracker dye, which labels mitochondria and plastids, to localize those organelles during this part of the cell cycle. By comparing the distribution of these organelles we demonstrate that BY-2 cells can sort their organelles during mitosis and assign them to specific positions within the phragmosome. Finally we demonstrate that cytoskeletal elements play only a limited role in maintaining the specific Golgi stack localization.

## RESULTS

### Golgi Stacks Accumulate near Spindle Poles and in an Equatorial Belt during Metaphase

Attempts at synchronizing the GmMan1-GFP-expressing cells by aphidicholin treatment (Nagata et al., 1982) were met with limited success (mitotic index approximately 15%), which may be due to the slightly lower growth rate of the transgenic line when compared with untransformed BY-2-cells (data not shown). We did not try to increase the mitotic index by an additional propyzamide treatment (Samuels et al., 1998) since the disruption of MTs can be expected to alter the cellular organization at the onset of mitosis. The results presented here therefore were derived from unsynchronized cultures that were not exposed to any drugs, unless indicated. Observations were conducted 2 to 4 d after transfer of the cells to fresh medium, when the number of dividing cells was relatively high (mitotic index approximately 6%).

During interphase, Golgi stacks appeared to be randomly distributed throughout the cortical and perinuclear cytoplasm (Fig. 1A; Nebenführ et al., 1999). The distribution of Golgi stacks in different parts of the cytoplasm was analyzed in three-dimensional image stacks of three interphase cells using an automated peak-finding algorithm to identify stacks followed by manual corrections. Identification of Golgi stacks in the cells was complicated by the dynamic nature of stack localization and limitations of the optical equipment. We have therefore analyzed only the upper one-half of each cell where the Golgi stacks



**Figure 1.** Stereo images showing the Golgi stack distribution in a living tobacco BY-2 cells in interphase (A) and metaphase (B). A, Interphase cell. Projections of 30 individual deconvolved epifluorescence images taken at 1- $\mu\text{m}$  intervals. N, Nucleus; w, cell wall. B, Metaphase cell. Projections of a three-dimensional reconstruction derived from 60 individual deconvolved epifluorescence images taken at 0.5- $\mu\text{m}$  intervals. Arrows denote the location of the spindle poles. The dashed line traces an equatorial accumulation of Golgi stacks, the "Golgi belt". Animated three-dimensional reconstructions of these cells can be viewed at <http://mcdcb.colorado.edu/~nebenfue/cytokinesis>.

were more easily identifiable. The numbers therefore represent approximations that illustrate the pattern of Golgi stack distribution within some unknown margin of error. In this way between 600 and 1,200 Golgi stacks were detected in the cells. The interphase cells analyzed had approximately two-thirds of their Golgi stacks in the cortical cytoplasm. Only

about one-tenth of all stacks were found in the perinuclear cytoplasm with the remainder (about one-fifth) being localized to the transvacuolar strands (Table I).

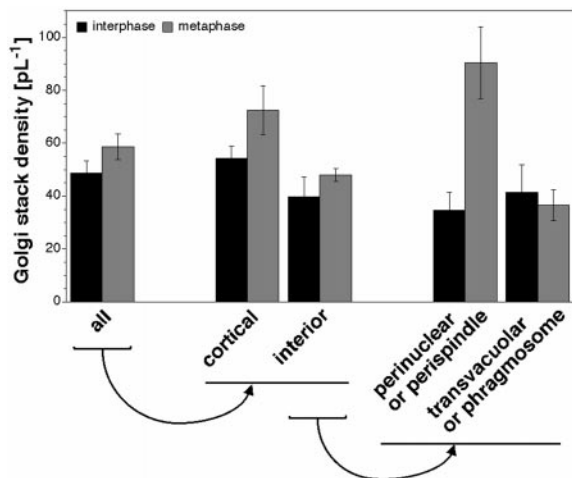
To test whether this distribution of Golgi stacks is truly random we have correlated the number of Golgi stacks in different regions of the cells with the available volume of cytoplasm. For this purpose, cytoplasm was defined as the area covered by the combined fluorescence of our GFP-marker, which stains the Golgi and to some extent, the ER (Nebenführ et al., 1999), and the MitoTracker dye, which stains mitochondria and plastids (see below). An additional background subtraction largely eliminated the diffuse ER-like fluorescence. This approach excludes cytoplasmic domains that do not contain these larger organelles, such as very thin layers of the cortical cytoplasm, the nucleoplasm, or the mitotic spindle. The absolute values of cytoplasmic volume obtained in this way therefore should be considered approximations and be interpreted with caution. However, the relative distribution of cytoplasm within cells can be expected to average out these uncertainties and thus constitute a relatively robust measure. The overall distribution of cytoplasm in the three interphase cells analyzed closely resembles the distribution of Golgi stacks (Table I). When the numbers of Golgi stacks found in the different regions of these cells were divided by the approximate cytoplasmic volumes in the respective regions, no statistically significant difference could be found between cortical and interior, or between perinuclear and transvacuolar cytoplasm (Fig. 2, black bars). Thus the distribution of Golgi stacks in interphase cells reflects the distribution of cytoplasm and appears to be random.

As the cells prepared for mitosis, the nucleus migrated to a central position in the cell. At this stage, large amounts of cytoplasm accumulated in a perinuclear location, forming a so-called "phragmosome" (Lloyd, 1991). The nucleus was usually displaced to one side, almost touching the plasma membrane. On the opposing side, the phragmosome was connected to the adjacent cortical cytoplasm by one or a few thick cytoplasmic strands. Most other transvacuolar strands of cytoplasm, on the other hand, disappeared. During early mitotic stages, most Golgi

**Table I.** Distribution of Golgi stacks in cortical and internal cytoplasm during interphase and metaphase

Golgi stack distributions were derived from direct counting of stacks and manual outlining of relevant regions. Cytoplasmic volume was defined as the area covered by the combined GFP and MitoTracker fluorescence, i.e. it represents the part of the cytosol accessible to larger organelles. Values are arithmetic means  $\pm$  SE,  $n = 3$ .

Interphase Cells			Metaphase Cells		
Location	Golgi stacks	Cytoplasm	Location	Golgi stacks	Cytoplasm
	%			%	
Cortical cytoplasm	70 $\pm$ 4	62 $\pm$ 4	Cortical cytoplasm	54 $\pm$ 4	44 $\pm$ 4
Interior cytoplasm	30 $\pm$ 4	38 $\pm$ 4	Interior cytoplasm	46 $\pm$ 4	56 $\pm$ 4
Perinuclear	9 $\pm$ 4	12 $\pm$ 5	Spindle region	19 $\pm$ 3	13 $\pm$ 3
Transvacuolar	21 $\pm$ 3	26 $\pm$ 3	Transvacuolar	27 $\pm$ 6	42 $\pm$ 6



**Figure 2.** Golgi stack density in different regions of the cytoplasm in interphase (black bars) and metaphase cells (gray bars). Total numbers of Golgi stacks were determined with an automated peak-finding algorithm. Cytoplasmic volume in manually delineated regions of the cells was defined as the area covered by the combined GFP and MitoTracker fluorescence, i.e. the part of the cytosol accessible to larger organelles. Golgi stack density is given as number of stacks per picoliter of cytoplasm. Error bars represent the SE ( $n = 3$ ). Note the much higher density of Golgi stacks in the immediate vicinity of the spindle in metaphase cells.

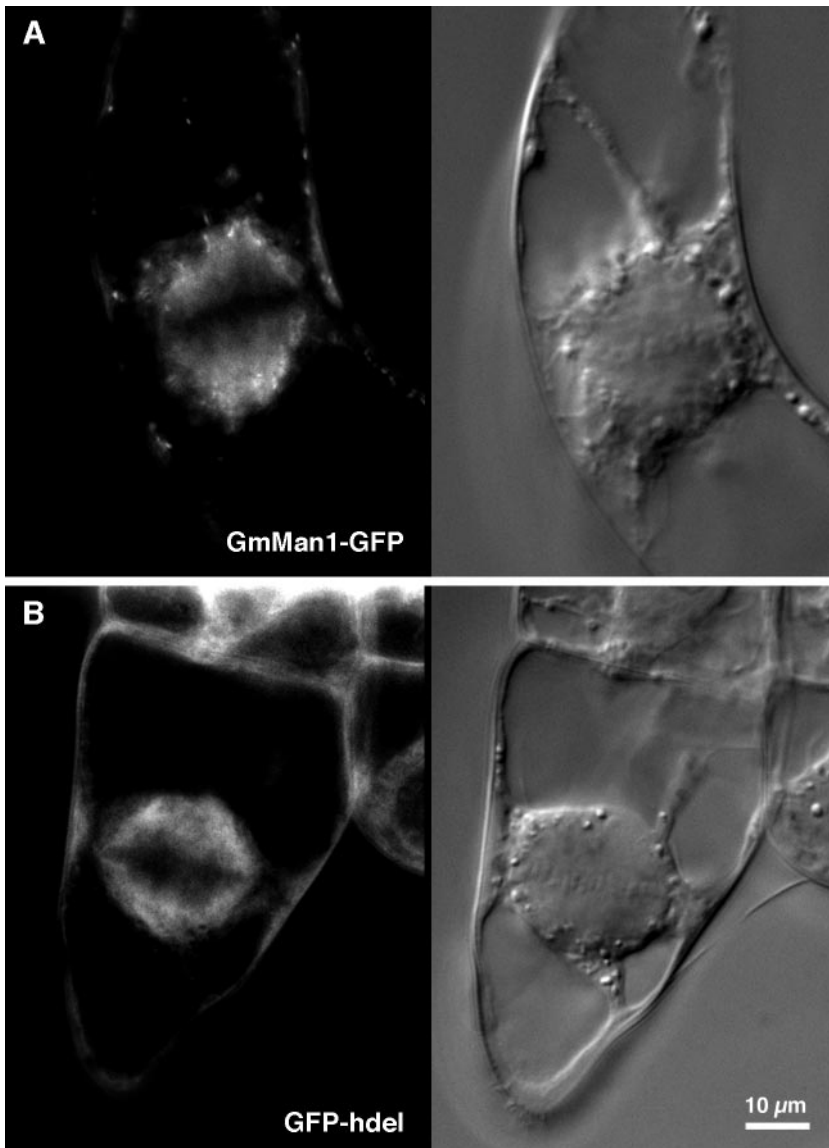
stacks accumulated in the phragmosome region, leaving the cortical cytoplasm depleted of Golgi stacks. The redistribution of Golgi stacks into the phragmosome did not involve the rapid stop-and-go movements described for interphase cells (Nebenführ et al., 1999), which could not be observed during any of the mitotic and cytokinetic events. When the cells reached metaphase, the Golgi distribution within the phragmosome underwent further changes. In particular, a large number of Golgi stacks accumulated around the spindle MTs and the spindle poles (Figs. 1B, arrows and 3A). The number of stacks tightly associated with the mitotic spindle was quantified in three-dimensional image stacks of three cells in metaphase. Thus about 20% of all stacks in the cells analyzed were localized in immediate proximity to the metaphase spindle (Table I). This number may be an underestimate since the selected spindle area was chosen conservatively to ensure that cortical stacks (in the Golgi belt) and those in other parts of the phragmosome would not be included. A slightly larger number of stacks was found in the rest of the interior cytoplasm, leaving approximately 55% ( $\pm 4\%$ , SE) of all stacks in the cortical cytoplasm.

Further quantitative analysis of metaphase cells demonstrated that the distribution of Golgi stacks did not match the distribution of cytoplasm as well as in the interphase cells (Table I). In particular, the phragmosome outside the spindle region accounted for  $42\% \pm 6\%$  of the cytoplasm, but it contained only  $27\% \pm 6\%$  of all Golgi stacks. In contrast, the imme-

mediate vicinity of the metaphase spindle represented only  $13\% \pm 3\%$  of the total cytoplasm, but contained  $19\% \pm 3\%$  of the Golgi stacks (Table I). This difference is readily apparent when the Golgi stack densities are plotted for the different cellular regions (Fig. 2, gray bars). The perispindle region of the interior cytoplasm had a more than 2-fold higher density of Golgi stacks than the rest of the phragmosome and transvacuolar strands ( $90 \pm 14 \text{ pL}^{-1}$  versus  $37 \pm 6 \text{ pL}^{-1}$ ). We expect this difference, although as such statistically not significant (paired  $t$  test,  $P > 0.05$ ), due to the small sample size, to persist when a more robust method of assessing cytoplasmic volume is developed. In contrast, the slightly higher Golgi stack density in the cortical cytoplasm may disappear with better measurements of cytoplasmic volume. Our data, despite their inherent uncertainties, therefore suggest that the distribution of Golgi stacks during metaphase is not random.

Most metaphase cells also accumulated Golgi stacks in a narrow, band-like region of cortical cytoplasm around the edges of the metaphase plate, which we have termed the "Golgi belt" (dashed line in Fig. 1B). This Golgi belt region appears to correspond to the site of the former preprophase band (PPB) of MTs since it coincides with the future site of cell division (see below). We have quantified the accumulation of Golgi stacks within the Golgi belt by counting the number of stacks in a cortical layer of cytoplasm in the same three cells at metaphase. For these experiments, the Golgi belt area was defined visually and individual stacks were identified manually. Only stacks immediately underlying the plasma membrane were analyzed to avoid complications due to uneven thickness in the cortical cytoplasm. The Golgi belt covered between 12% and 20% of the cell surface and the average density of stacks within the belt region was about 3-fold higher than in the rest of the cortical cytoplasm. In two cases we were able to determine the overall stack density in the cortical cytoplasm for two adjacent sister cells in metaphase and interphase, respectively. In both pairs of cells, the metaphase sister had a lower density of Golgi stacks in the cortical cytoplasm than the interphase sister (75% and 80% of the interphase sister, respectively). This difference was more pronounced in the peripheral cortex (i.e. the cortex excluding the Golgi belt in metaphase and the perinuclear cortex in interphase) where the Golgi stack density dropped to about 60% of the interphase value during metaphase, suggesting that about one-third of the cortical Golgi stacks in the mitotic cells had been relocated to the phragmosome.

Although we attempted to eliminate variations in stack distribution due to changes in the thickness of the cortical cytoplasm, we cannot exclude the possibility that in some regions the space available between the plasma membrane and the tonoplast



**Figure 3.** Comparison of GmMan1-GFP (A) and GFP-hdel (B) fluorescence in metaphase cells. On the left are fluorescence images and on the right corresponding differential interference contrast (DIC) image of the same cells. A, BY-2 cell expressing GmMan1-GFP. Note the presence of hazy, string-like fluorescence in the mitotic spindle in addition to the punctate appearance of Golgi stacks. B, BY-2 cell expressing ER-targeted GFP (GFP-hdel). A similar hazy fluorescence pattern is seen in the spindle.

was too small to accommodate larger organelles such as Golgi stacks. We have therefore analyzed the cortical distribution of mitochondria, which usually are only slightly smaller than Golgi stacks in BY-2 cells, in the same cells. Mitochondria were stained with the MitoTracker dye that partitions preferentially into membranes with high membrane potential (Haugland, 1996). In plants these are mitochondria and plastids, which can be easily distinguished based on their different sizes. Again, only organelles immediately underlying the plasma membrane were considered. Although mitochondria also show a slight accumulation in the region of the Golgi belt, this preferential localization was much smaller than that found for Golgi stacks (Table II). We conclude that the apparent accumulation of Golgi stacks in the Golgi belt is not an artifact of differential cytoplasmic accumulation, but that it reflects the specific concentration of this organelle around the cell equator.

**Table II.** Density of Golgi stacks and mitochondria in the cortical cytoplasm during metaphase

Individual stacks or mitochondria immediately underneath the plasma membrane were marked manually. Different regions of the cortical cytoplasm were identified by eye and outlined. Values are arithmetic means  $\pm$  SE ( $n = 3$ ) and given in  $\mu\text{m}^{-2}$ .

Location	Organelle Density	
	Golgi stacks	Mitochondria
	$\mu\text{m}^{-2}$	
Overall	$0.09 \pm 0.01$	$0.13 \pm 0.02$
Belt region	$0.17 \pm 0.02$	$0.16 \pm 0.03$
Peripheral cortex	$0.06 \pm 0.01$	$0.11 \pm 0.02$
Concentration in belt over periphery	$2.9 \pm 0.3$	$1.5 \pm 0.4$

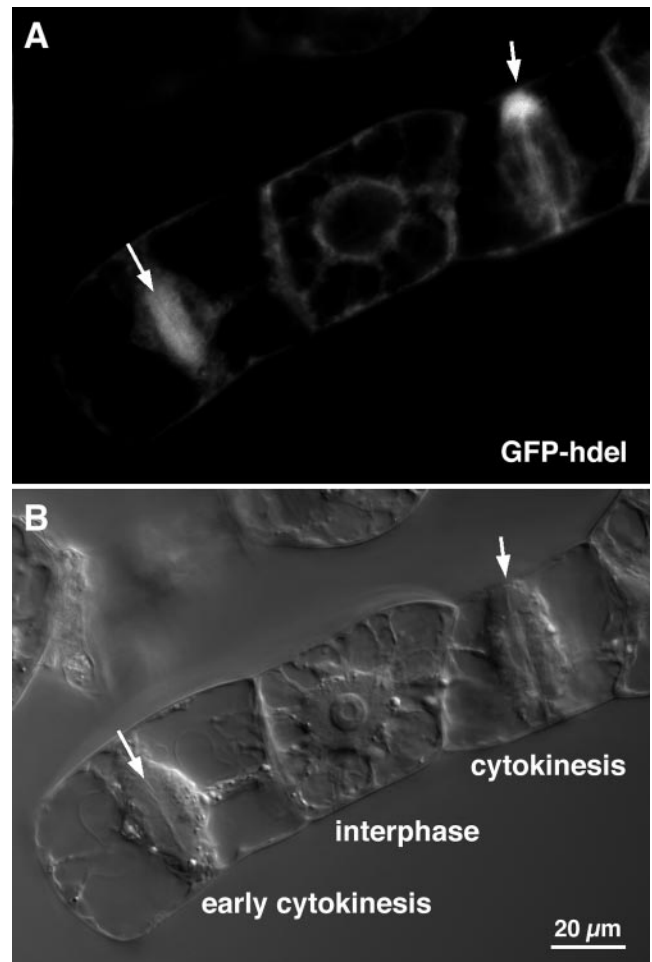
### Diffuse Fluorescence during Mitosis May Result from Partial ER Localization of the cis-Golgi Marker

During interphase, the GFP fluorescence was largely restricted to Golgi stacks, although a fraction of the fusion proteins could be detected in the ER (Nebenführ et al., 1999). When the cells entered mitosis, the fluorescence appeared to become more diffuse (compare Fig. 1, A and B) and also spread to areas that excluded Golgi stacks such as the spindle region (Fig. 3A). The latter localization indicates the presence of the GmMan1-GFP fusion protein in the ER. This interpretation is supported by observations of cells expressing GFP in the lumen of the ER, which showed a similar distribution of fluorescence (Fig. 3B). In particular, the ER marker was redistributed into the phragmosome, leaving only small amounts of ER in the cortical cytoplasm and transvacuolar strands (compare Fig. 3, B and 4, central cell). Within the phragmosome, the highest ER density was found near the spindle poles at metaphase (Fig. 3B), and later in the region of the phragmoplast (Fig. 4, outer cells), thus confirming earlier reports in the literature for other cell types (Hepler, 1980, 1982; Schopfer and Hepler, 1991). The apparent increase in ER-like fluorescence in dividing cells expressing GmMan1-GFP raises the possibility that our Golgi marker partially relocates into the ER during mitosis and cytokinesis. To address this question we have attempted to quantify the relative level of diffuse ER-like versus punctate Golgi fluorescence in optical sections through the center of the cell. However, the intermingling of the two organelles made it impossible to reliably quantify the contributions of the two organelles independently.

### Golgi Stacks Are in Close Proximity to the Phragmoplast during Cytokinesis

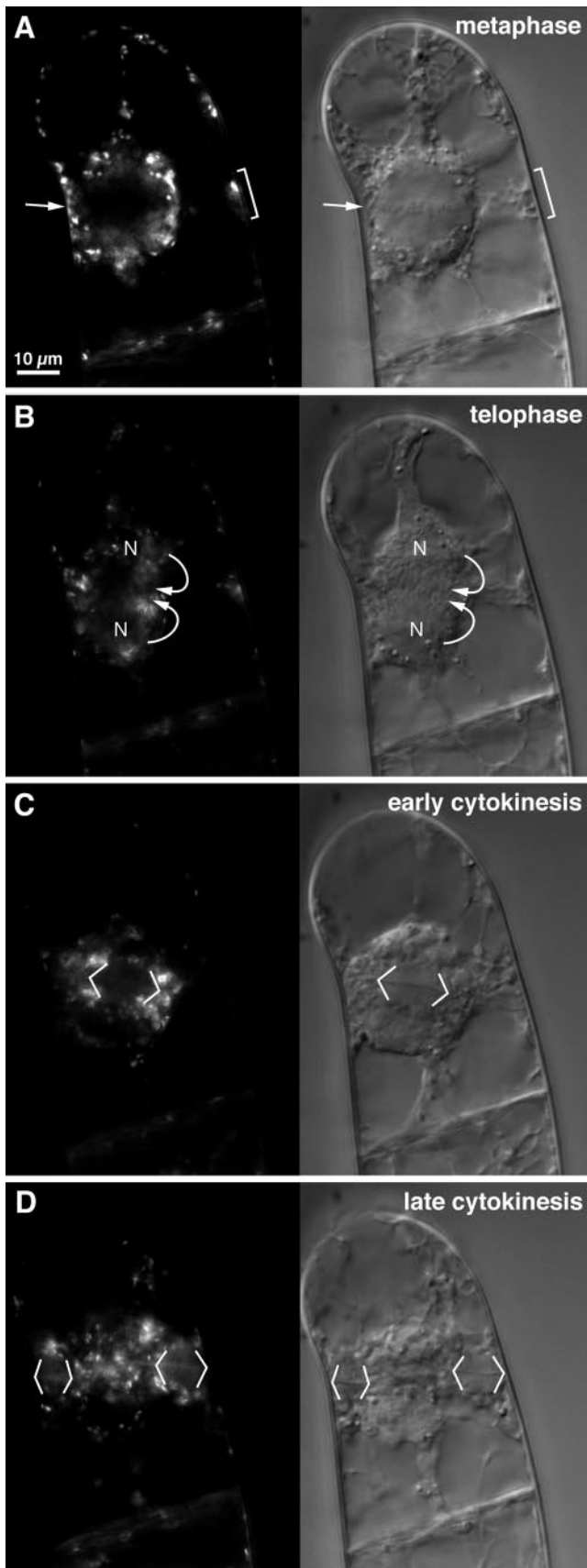
As the cells progressed into ana- and telophase, individual stacks appeared in the region between the daughter nuclei (Fig. 5, A and B). After phragmoplast formation, Golgi stacks were excluded from the region of high cytoskeletal density (Fig. 5, C and D). Only diffuse, ER-like fluorescence was visible in the phragmoplast, whereas Golgi stacks became enriched in the surrounding cytoplasm (Fig. 5, C and D). In many cells, the daughter nuclei flattened out and approached the new cell plate while the phragmoplast was growing centrifugally. During the outward growth of the phragmoplast, Golgi stacks were continuously associated with the periphery of the cytoskeletal elements. In addition, stacks could be found in close proximity to the more mature cell plate in the center of the cell (Fig. 5D).

In many cells the nucleus assumed an asymmetrical position prior to mitosis, which often resulted in the cell plate reaching one parental wall long before the other. Careful observation of Nomarski images,



**Figure 4.** Distribution of ER in interphase and cytokinetic in GFP-hdel expressing BY-2 cells. A, Fluorescence image showing the distribution of the GFP-hdel marker. B, DIC image of the same group of cells. The left cell is in telophase/early cytokinesis with a prominent phragmoplast between the daughter nuclei. The right cell is in a later stage of cytokinesis with a phragmoplast near to top edge of the cell. The central cell is in interphase. Arrows point at the forming cell plates within the phragmoplasts. The strong GFP-hdel fluorescence within the phragmoplasts reveals the abundance of ER in this region. Note the change in ER distribution between interphase and cytokinesis. The strong labeling of cortical cytoplasm and transvacuolar strands during interphase is nearly absent in dividing cells.

however, revealed that in such instances a phragmoplast was present on both sides of the cell immediately prior to fusion of the cell plate with the plasma membrane (Fig. 5D and data not shown). This suggests that the cell plate fuses with the plasma membrane nearly simultaneously all around the cell. Late stage phragmoplasts in BY-2 cells typically had a width of approximately 10  $\mu\text{m}$  when viewed with Nomarski optics (Fig. 5D). The entire process from ana-/telophase to completion of the cell plate was accomplished within 90 min. Time-lapse video analysis of individual cells maintained in a perfusion chamber revealed that Golgi stacks continuously “wiggled” and shifted positions within the phragmosome while



maintaining their general spatial relationship to the phragmoplast (compare time-lapse video sequence at <http://mcdb.colorado.edu/~nebenfue/cytokinesis>).

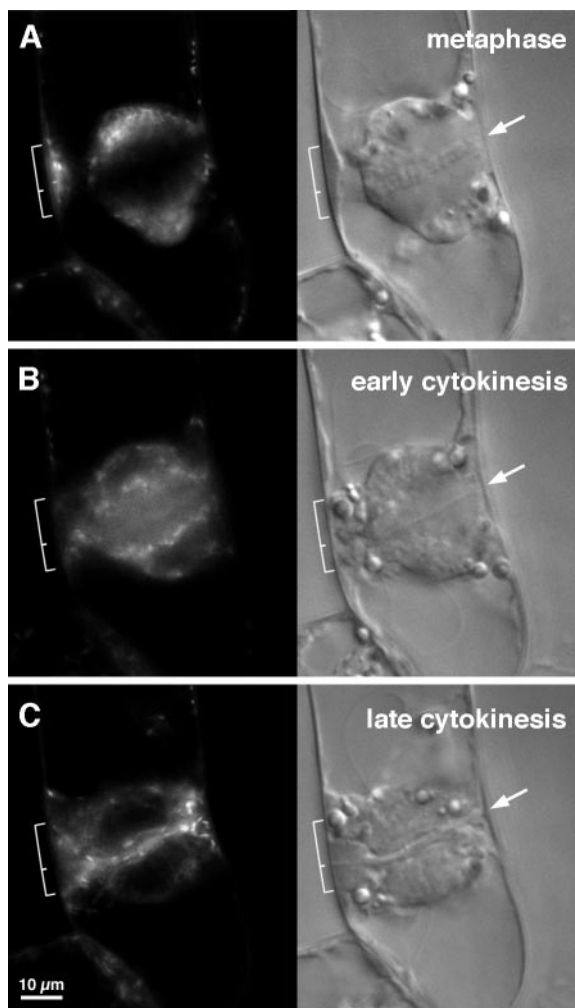
It has been shown for mammalian cells that the distribution of mitotic Golgi fragments into daughter cells is very close to the predicted 1:1 ratio (Shima et al., 1997, 1998). We have determined the distribution of Golgi stacks in pairs of daughter cells to test whether BY-2 cells with their much larger number of individual stacks also partition their Golgi apparatus evenly. Analysis of three pairs of cells immediately before or after completion of the cell plate revealed that the daughter cells did not receive identical numbers of Golgi stacks (ratio 1:1.16  $\pm$  0.10). However, it was evident that the two cells usually were of different size, with the larger of the two receiving more stacks. We have therefore determined the approximate volume of cytoplasm in the two daughter cells as either the area covered by the combined fluorescence of GmMan1-GFP and MitoTracker (see above) or as the total cell volume minus that of the manually delineated vacuole in three-dimensional DIC image stacks. The difference in normalized Golgi stack densities between pairs of cells was found to be only 9% ( $\pm$ 7%). Thus, the Golgi stacks in dividing BY-2 cells appear to be approximately evenly distributed into the cytoplasm of the resulting daughter cells.

#### Cell Plates Grow toward Surface Regions Defined by the Golgi Belt

As described earlier, Golgi stacks accumulated in an equatorial belt underlying the plasma membrane during metaphase (Fig. 1B). We have examined the spatial relationship of this Golgi belt with other landmarks and events during mitosis and cytokinesis to gain some insight regarding its possible function. The early phragmoplast is assembled in the region of the mitotic spindle during ana- and telophase, and the pole MTs are thought to provide the initial guidance (Lambert et al., 1991). The phragmoplast

**Figure 5.** Time-lapse observation of Golgi stack distribution in a dividing BY-2 cell. Fluorescence (left) and DIC images (right) were taken in close succession at various time points during mitosis and cytokinesis. A, Metaphase. Arrow denotes plane of metaphase plate of chromosomes. Bracket indicates the position of the “Golgi belt” in the cortical cytoplasm. B, Telophase. The daughter nuclei (N) have moved out to the spindle poles and Golgi stacks are seen entering the internuclear region (arrows). C, Early cytokinesis. The phragmoplast (angle brackets) has formed between the daughter nuclei. Golgi stacks are excluded from the phragmoplast but accumulate in its vicinity. D, Late cytokinesis. The phragmoplast (angle brackets) has grown centrifugally and has almost reached the parental plasma membrane. Golgi stacks, but not ER, continue to be excluded from the phragmoplast but are present in close proximity. Note the presence of Golgi stacks close to the more mature cell plate in the center of the cell. A time-lapse video sequence of this cytokinesis can be viewed at <http://mcdb.colorado.edu/~nebenfue/cytokinesis>.

thus maintains the same orientation as the mitotic spindle, and formation of the initial (internuclear) cell plate occurs in the same plane as the metaphase plate of chromosomes (compare Fig. 5, A and C). We have observed eight cells in which the metaphase plate was oriented at an angle relative to the Golgi belt (Fig. 6A). In these cases the early cell plate was also created in the same plane as the metaphase chromosomes (Fig. 6B). However, when the phragmoplast left the interzone between the daughter nuclei, it changed direction leading to a kink in the immature cell plate (Fig. 6C). The reorientation of the phragmoplast always occurred toward the area of Golgi stack accumulation identified in metaphase, and cell plate fusion with the parental plasma membrane was always in the region of the Golgi belt



**Figure 6.** Time-lapse series demonstrating that the Golgi belt predicts the site of cell plate fusion with the plasma membrane. Fluorescence images (left) and DIC images (right) of a BY-2 cell with a slanted metaphase plate, taken at metaphase (A), telophase/early cytokinesis (B), and late cytokinesis (C). Note that the early cell plate appears in the same plane as the metaphase plate (arrows). Later the left end of the phragmoplast curves upwards toward an area of Golgi stack accumulation, the Golgi belt (bracket).

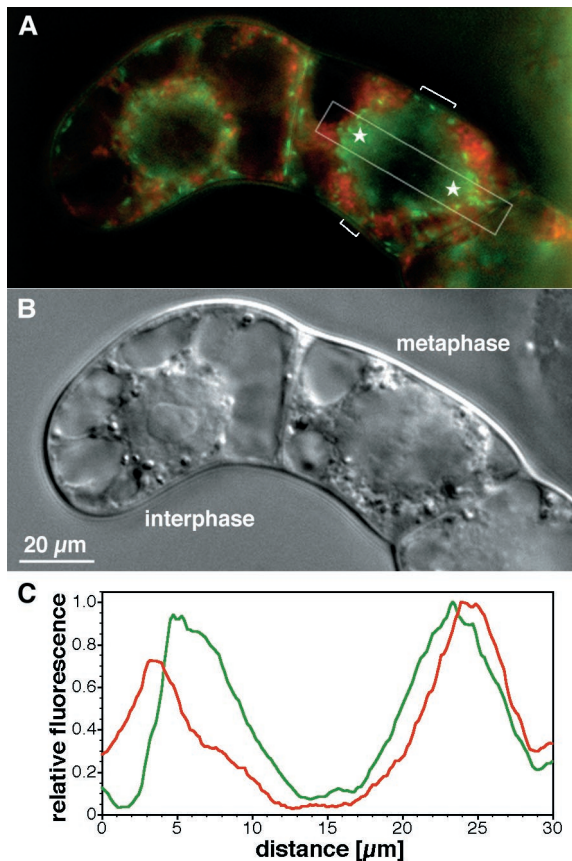
(brackets in Fig. 6). Thus the Golgi belt appears to accurately predict the future site of cell division after the disassembly of the PPB of MTs (Mineyuki, 1999).

#### Regions of Golgi Stack Accumulation Exclude Mitochondria and Plastids

We have shown that the distribution of Golgi stacks during metaphase is not random and does not follow the distribution of cytoplasm (Fig. 2; Table I). The observed accumulation of stacks in the Golgi belt is specific for this organelle and is not mimicked by mitochondria (Table II). It is unfortunate that we were not able to perform a similar analysis in the cell interior since the high density of mitochondria within the phragmosome together with limitations in our optical equipment precluded us from reliable identification of individual organelles in this region. Nevertheless we were able to correlate the Golgi stack localization to the distribution of mitochondria and plastids in living cells. In interphase cells, the red fluorescence of the MitoTracker dye and the green fluorescence of the GFP tag was intermingled and showed no particular arrangement (Fig. 7A, left cell). In contrast, during metaphase, the two sets of organelles marked by the two labels partitioned into complementary cellular regions (Figs. 7A, right cell and 8A). In particular, whereas the Golgi-specific and ER-like green fluorescence were seen preferentially around the spindle poles, in the Golgi belt, and in the spindle, the red fluorescence showed that mitochondria and plastids accumulated outside the spindle and in the cortical cytoplasm outside the Golgi belt (Figs. 7A, right cell and 8A). Analysis of the fluorescence intensities in a 5- $\mu\text{m}$ -wide strip that stretched across the phragmosome and the spindle poles revealed that the green GFP fluorescence marking Golgi stacks and ER peaked several  $\mu\text{m}$  (range of 1–5  $\mu\text{m}$ ) closer to the spindle than the mitochondria and plastid-specific fluorescence (Fig. 7C).

During cytokinesis, this differential distribution of organelles was largely maintained (Fig. 8, B and C). Again, diffuse ER-like fluorescence was detected in the region of the phragmoplast, whereas the stronger and more particulate staining of the Golgi stacks was evident in the perinuclear cytoplasm, also between the daughter nuclei and the phragmoplast, as well as in the Golgi belt (Fig. 8B). Plastids were not present in these regions, whereas the smaller mitochondria could approach the phragmoplast more closely (Fig. 8, B and C). During late cytokinesis, a few mitochondria were evident in the region between the nuclei and the phragmoplast/cell plate, whereas the larger plastids were completely excluded from this region, usually remaining on the far side of the daughter nuclei (Fig. 8C).





**Figure 7.** Comparison of fluorescence distribution for Golgi stacks/ER and mitochondria/plastids in adjacent interphase and metaphase cells. A, Merged fluorescence image showing Golgi stacks and ER in green and mitochondria and plastids (labeled with MitoTracker) in red. The stars denote the positions of the spindle poles. The brackets mark the region of the Golgi belt. B, DIC image of the same cells in interphase (left, note nucleolus) and metaphase (right, note metaphase plate), respectively.

### Golgi Stack Redistribution Commences in Metaphase

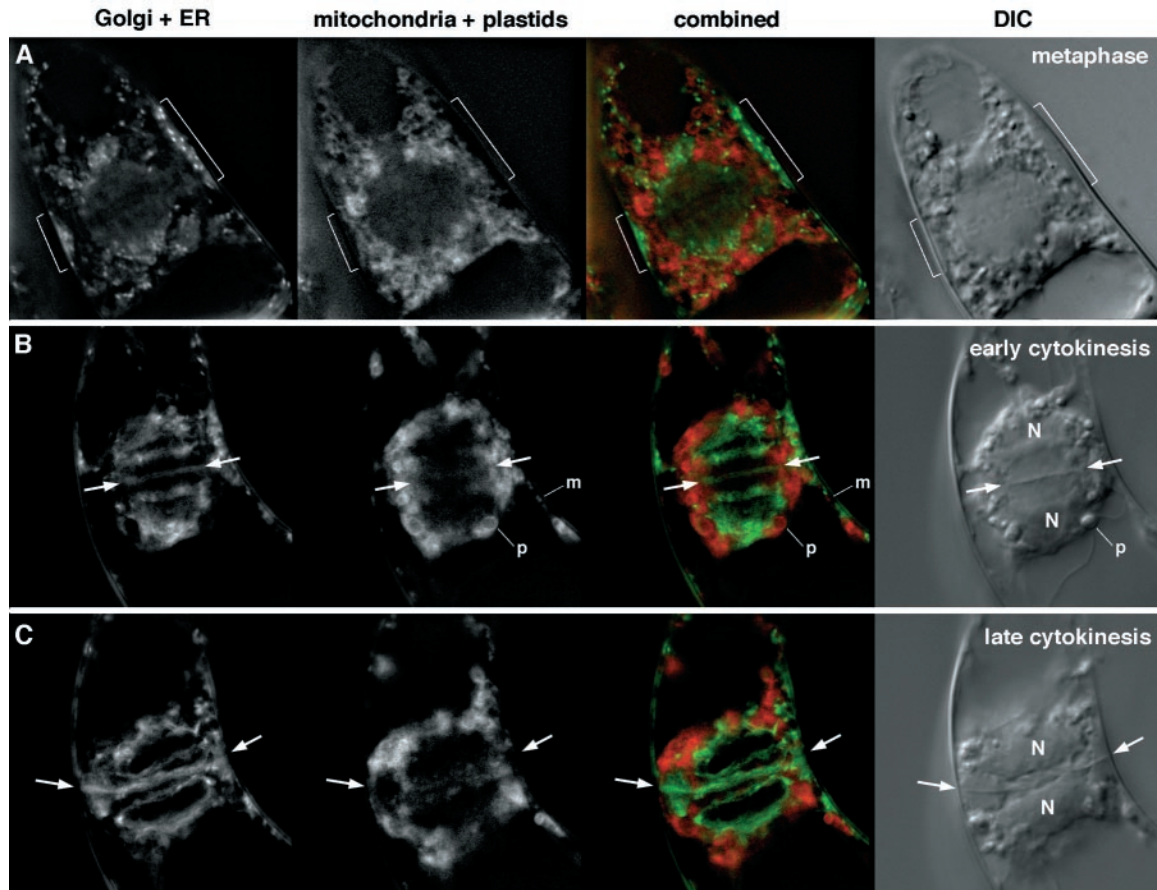
The results described so far indicate that plant cells segregate their organelles into distinct domains prior to metaphase, i.e. during early mitotic stages. To identify the precise time at which this segregation first becomes apparent, we have identified prophase cells by their lack of a nucleolus when viewed with Nomarski optics. Unfortunately, only a small number of cells could be identified in this way. At this stage, no Golgi stack accumulation near the prospective spindle poles was evident, and mitochondria and plastids were intermingled among the stacks (data not shown). However, all of these cells either reverted back to interphase during the observation period or failed to form a tight metaphase plate. We suspect that these effects were caused by the disruptive stress of observing the cells with high light intensities. To circumvent this problem we have fixed control BY-2 cells with 1% (v/v) glutaraldehyde and determined the arrangement of Golgi stacks in the

context of mitotic stage-defining MTs visualized by immunofluorescence (Fig. 9).

The dispersed distribution of Golgi stacks that is typical of interphase cells (Fig. 1A) was retained in prophase before the breakdown of the nuclear envelope (Fig. 9A). During prometaphase, when perinuclear MTs start to form a spindle, some cells displayed an increased number of Golgi stacks adjacent to the narrow PPB (Fig. 9B); however, not all cells at this stage exhibited this accumulation in the Golgi belt. The accumulation of Golgi stacks around the spindle poles described above (Figs. 1B and 7) was not evident in cells fixed during the early stages of spindle development (Fig. 9B). On the other hand, cells with fully formed mitotic spindles and a metaphase plate of chromosomes clearly displayed an aggregation of Golgi stacks near the spindle poles (Fig. 9C). In general, the distribution of Golgi stacks in cells fixed during or after metaphase was essentially the same as that found in living cells. For example, only a few Golgi stacks were found between the chromosomes and the forming phragmoplast in telophase (Fig. 9D). This number of internuclear Golgi stacks increased as the phragmoplast began to grow outwards (Fig. 9, E and F). In addition, Golgi stacks were found to accumulate near the phragmoplast during cytokinesis (Fig. 9, E and F). Thus it appears that the specific distribution of Golgi stacks observed in metaphase cells arises after breakdown of the PPB during the late stages of spindle formation. It should be noted, however, that the extensive processing of the samples for immunostaining may have led to some subtle rearrangements of organelles and cytoskeletal elements, making it more difficult to correlate precisely the living and the fixed cell images.

### Disruption of MTs Does Not Lead to a Mixing of Organelles

The specific localization of Golgi stacks and the removal of mitochondria and plastids from regions of Golgi stack accumulation was found to occur during the early stages of metaphase as the nuclear envelope breaks down and the spindle forms (Fig. 9, B and C). It is tempting to speculate that this redistribution of organelles is brought about by breakdown of the cortical MTs and formation of the mitotic spindle. To test this hypothesis we have treated cells undergoing mitosis with the MT-disrupting drug, propyzamide (6 μM). Analysis of living cells maintained in a perfusion chamber showed that although the drug produced the expected cell cycle arrest, the overall distribution of the Golgi stacks changed very little. In particular, the breakdown of the metaphase spindle allowed larger organelles to approach the chromosomes more closely (Fig. 10, compare A and B). However, the general segregation of Golgi stacks closer to the chromosomes and mitochondria and



**Figure 8.** Comparison of Golgi stack and ER (green) distribution with that of mitochondria and plastids (red) at various points during mitosis and cytokinesis. Each row shows from left to right green fluorescence channel, red fluorescence channel, merged fluorescence images, and DIC images. A, Metaphase; B, early cytokinesis; and C, late cytokinesis (same cell as in B). Brackets delineate the Golgi belt at metaphase. Arrows point at the growing ends of the cell plate. Note that in this case the late cell plate (C) curves upward toward regions that were rich in Golgi stacks earlier (B). N, Nucleus; p, plastid; m, mitochondrion.

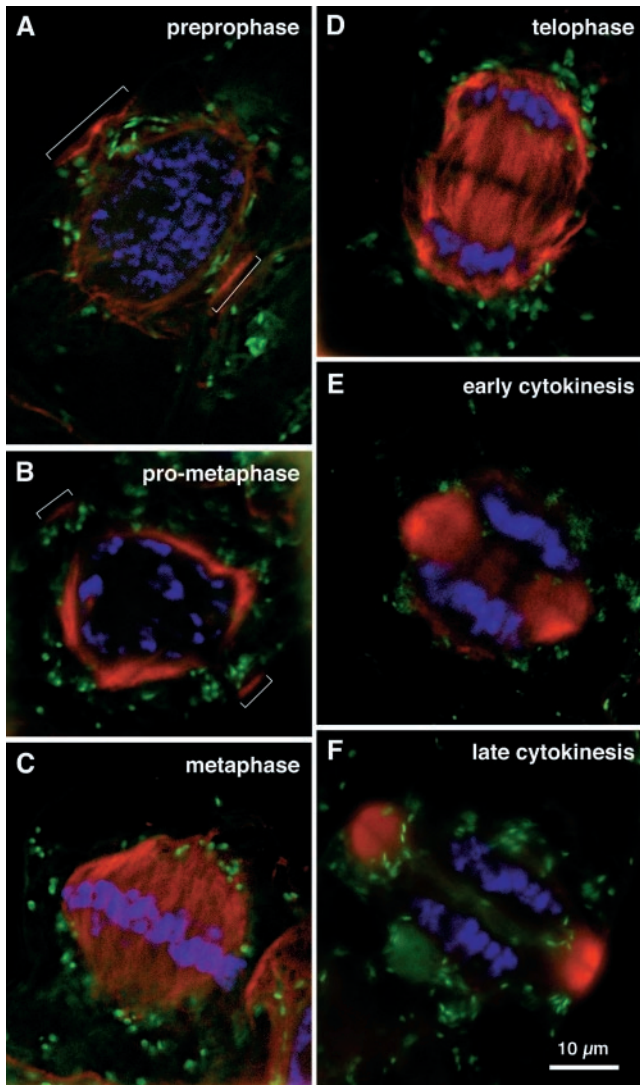
plastids in the periphery of the phragmosome was largely maintained.

It should be noted that under these conditions the spindle usually did not disappear completely (Fig. 10B), which presumably reflects the insensitivity of kinetochore fibers to MT-disrupting drugs (Binarová et al., 1998). If, on the other hand, the cells were treated with propyzamide under continuous agitation, the spindle collapsed completely and large organelles were directly opposed to the condensed chromosomes (Fig. 10C). We speculate that the few kinetochore MTs left after propyzamide treatment are not sufficient to resist the strong physical forces in tumbling cells. Under these conditions, the segregation of Golgi stacks, mitochondria, and plastids was partially lost although still recognizable (arrowheads in Fig. 10C), suggesting that MTs are not absolutely required for maintenance of organelle segregation. At the same time, however, this result demonstrates that the physical strains imposed on tumbling cells in agitated cultures can affect the cell

architecture in the absence of stabilizing cytoskeletal elements.

#### Golgi Stacks Do Not Appear to Require Actin MFs for Maintaining Their Specific Localization

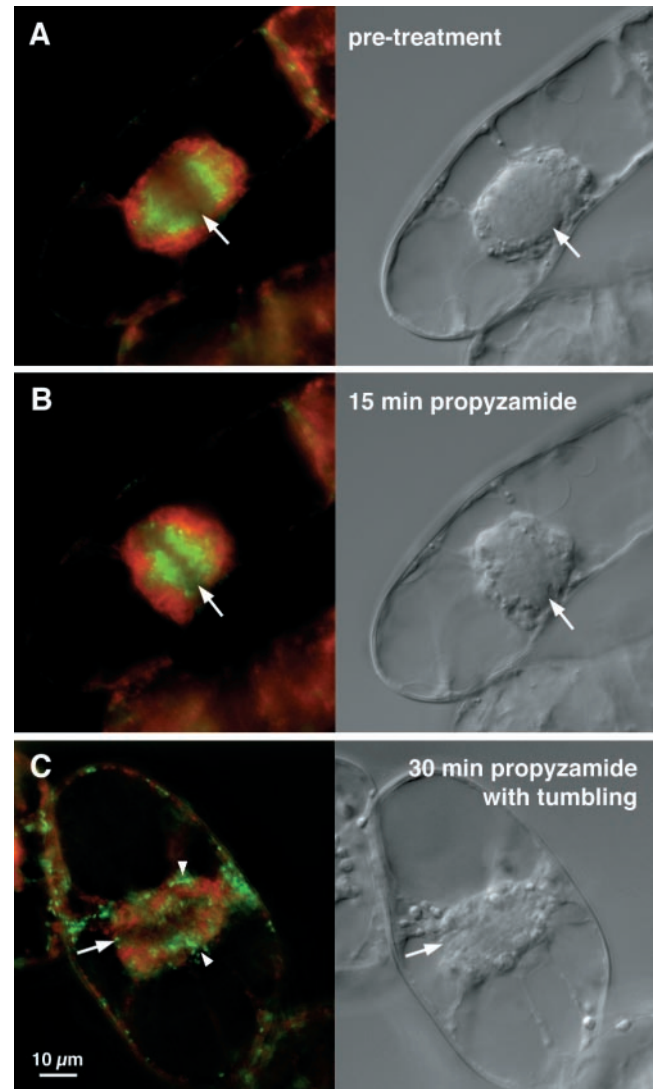
We have shown previously that Golgi stacks can travel along actin filaments in interphase BY-2 cells (Nebenführ et al., 1999). This raises the possibility that Golgi stack distribution during mitosis and cytokinesis also depends on actin filaments. We have therefore examined the spatial relationship between Golgi stacks and actin filaments in fixed cells, as well as the effect of actin-disrupting drugs on Golgi distribution in dividing BY-2 cells. Control cells were fixed and stained for actin with an anti-pea actin monoclonal antibody (Andersland et al., 1994). In metaphase cells, thick actin filaments were found throughout the cortical cytoplasm, in transvacuolar strands, and surrounding the mitotic spindle (Fig.



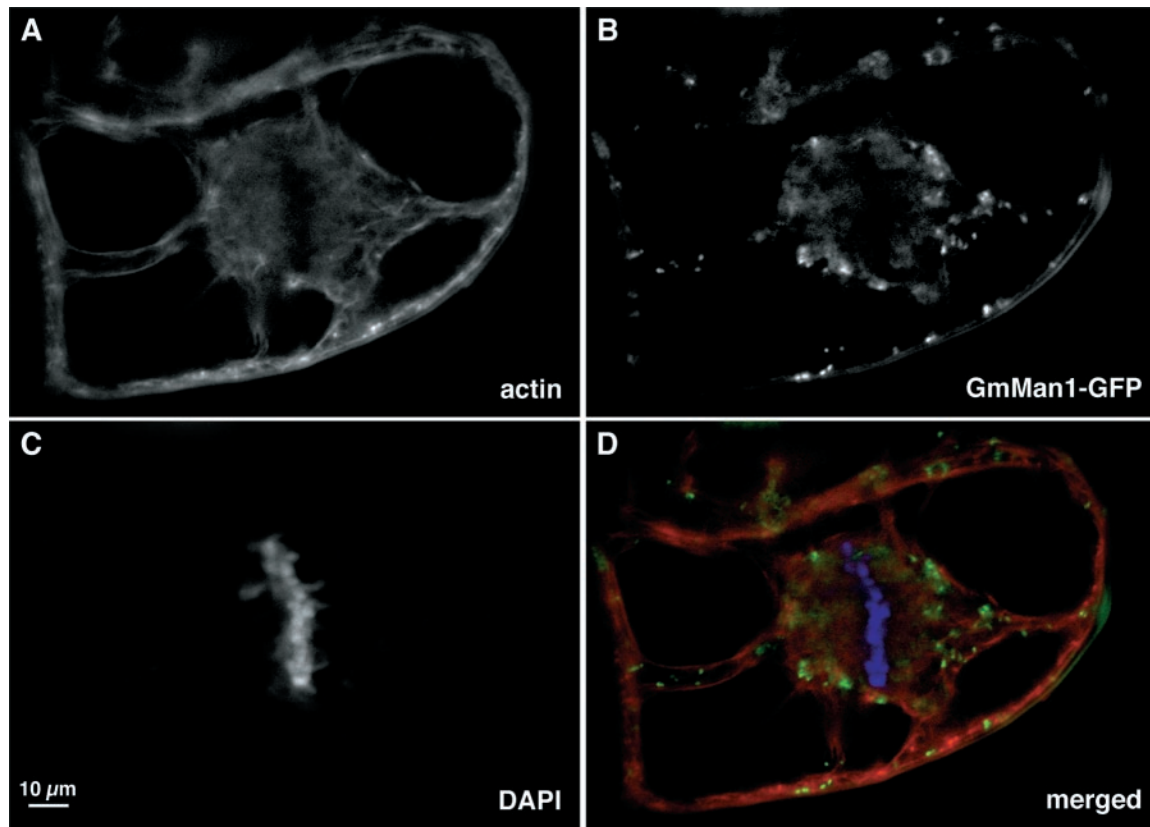
**Figure 9.** Golgi stack distribution relative to MTs. MTs (red) were detected in fixed BY-2 cells by indirect immunofluorescence. DNA (blue) was marked with DAPI, and Golgi stacks are green. A, Preprophase. Note the condensing chromosomes and the broad PPB of MTs (brackets). No particular arrangement of Golgi stacks can be discerned. B, Pro-metaphase. Chromosomes are fully condensed. The spindle is forming and the PPB (brackets) has almost disappeared. Golgi stacks show some accumulation near spindle poles and PPB/Golgi belt. C, Metaphase. Spindle is fully formed and chromosomes are arranged in metaphase plate. Golgi stacks are accumulated at spindle poles; the Golgi belt is not visible in this cell. D, Telophase. Chromosomes have moved to spindle poles and phragmoplast is starting to form in the interchromosomal region. Few Golgi stacks can be found between the MTs. E, Early cytokinesis. The phragmoplast has mostly disappeared from the center, allowing a few Golgi stacks into the internuclear region. F, Late cytokinesis. The phragmoplast has almost reached the plasma membrane. Golgi stacks are present close to the phragmoplast as well as surrounding the daughter nuclei.

11A). This latter distribution near the spindle matches that of Golgi stacks (Fig. 11B). However, only a few Golgi stacks could be clearly localized to actin fila-

ments within the phragmosome (Fig. 11D). Disruption of actin filaments with either 20 μM cytochalasin D or 1 μM latrunculin A did not lead to a redistribution of Golgi stacks in mitotic living cells (data not shown). Similarly, the segregation of Golgi stacks (and ER) from mitochondria and plastids was not affected in cells treated with the actin-filament disrupting drugs. However, the treated cells became arrested in their given stage of mitosis or cytokinesis,



**Figure 10.** Effect of MT-disrupting drugs on organelle distribution. A and B, Individual BY-2 cell in metaphase before (A) and after a 15-min treatment with 10 μM propyzamide (B). The cell was treated in a perfusion chamber on the microscope stage. Arrow denotes the plane of the metaphase plate of chromosomes. Note the separation of Golgi and ER (green) and mitochondria and plastids (red) that is maintained even after the spindle has partially collapsed. C, BY-2 cell at metaphase that has been treated with 10 μM propyzamide for 30 min under constant agitation. The spindle has completely collapsed and the organelles have closely approached the chromosomes (arrow). Note that green fluorescent Golgi stacks are clustered near the center of the metaphase plate (arrowheads).



**Figure 11.** Golgi stack distribution relative to actin filaments in a metaphase cell. Actin filaments (red) were detected in fixed BY-2 cells by indirect immunofluorescence. A, Actin filaments (Cy3 channel). B, Golgi and ER distribution (fluorescein isothiocyanate channel). C, Chromosomes (DAPI channel). D, Merged fluorescence image showing actin (red) Golgi and ER (green) and chromosomes (blue). Thick actin filaments are found surrounding the phragmosome, in transvacuolar strands, and throughout the cortical cytoplasm. Thinner actin filaments are found throughout the phragmosome and inside the mitotic spindle. The Golgi stack distribution only partially matches that of the actin filaments.

making it impossible to observe the effects of the drugs on the redistribution of organelles. Thus actin filaments do not appear to be necessary for maintaining the specific distribution of Golgi stacks in dividing cells.

## DISCUSSION

Golgi stacks in plant cells are the sites of *de novo* synthesis of cell wall matrix polysaccharides, in addition to their role in sorting and modification of proteins and membrane lipids (Driouich and Staehelin, 1997; Andreeva et al., 1998; Dupree and Sherrier, 1998). Based on our analysis of the tightly regulated Golgi stack movement in interphase cells, we have speculated that the stacks can be recruited to locations where their products are needed (Nebenführ et al., 1999). The forming cell plate in dividing cells constitutes such a region of localized requirement for Golgi products and may therefore serve as a model with which to test the predictions of our hypothesis. In addition to playing a central role in cytokinesis, the Golgi stacks (together with the other organelles) should be distributed roughly equally among the

daughter cells to ensure optimal cell viability. In this study we have addressed both of these points by determining the distribution of Golgi stacks in living, dividing plant cells and comparing their distribution with that of mitochondria and plastids. Our results indicate that during mitosis and cytokinesis Golgi stacks are indeed sorted to specific areas of the cytoplasm that appear to be related to their sites of action.

### Plant Cells Sort Golgi Stacks, Mitochondria, and Plastids into Distinct Cytoplasmic Domains during Cell Division

We have used a Golgi-targeted fusion of GFP to soybean mannosidase to follow the redistribution of Golgi stacks during mitosis and cytokinesis in living tobacco BY-2 suspension culture cells. Throughout all stages of cell division, we observed an accumulation of Golgi stacks in the phragmosome region (e.g. Figs. 1B and 5). In particular we found that at metaphase, about 40% of the Golgi stacks accumulate in immediate vicinity of the spindle and in an equatorial belt underlying the plasma membrane, whereas interphase cells contain only 25% of their Golgi stacks in

the equivalent perinuclear and equatorial regions. Golgi stacks therefore are recruited into the phragmosome during early mitotic stages. It is not currently known whether this recruitment involves active movement of Golgi stacks along actin filaments or is the result of passive stack movement caused by the major reorganization of cellular architecture. Within the phragmosome, the stacks assume preferred positions, namely at the spindle poles and in a cortical belt that surrounds the mitotic machinery. This Golgi belt appears to predict the plane of cell plate fusion with the plasma membrane (Fig. 6) and thus constitutes a novel marker of the future division site after the disassembly of the PPB of MTs (Mineyuki, 1999). The Golgi belt may correspond to a reticulate band of cytoplasm underlying the plasma membrane in mitotic sycamore maple callus cells (Roberts and Northcote, 1970).

By comparing the distribution of Golgi stacks with that of mitochondria and plastids labeled with the MitoTracker dye we were further able to show that these organelles are largely localized to separate and complementary domains within the phragmosome. The regions of highest Golgi stack density at metaphase, the spindle poles and the Golgi belt, are completely devoid of plastids and contain only few mitochondria (Figs. 7 and 8). During cytokinesis, Golgi stacks are found in close proximity to, but not within, the phragmoplast (Figs. 5C and 9, E and F). Plastids are also absent from this region of Golgi stack accumulation, whereas some mitochondria can be found near the phragmoplast (Fig. 8, B and C). In the later stages of cytokinesis when the ring-shaped phragmoplast expands centrifugally, Golgi stacks and some mitochondria can approach the more mature parts of the cell plate, whereas plastids are still excluded from the region between the daughter nuclei and the cell plate (Fig. 8C).

To our knowledge, the striking, cytokinesis-related segregation of organelles in living plant cells documented in this study has not been described before. In the past, the distribution of organelles during mitosis and cytokinesis has received only little attention compared with the extensive literature on cytoskeletal rearrangements. For example, an accumulation of ER membranes near spindle poles was described for mitotic barley cells based on osmium tetroxide/potassium ferricyanide staining and observation in the electron microscope (Hepler, 1980). Similarly, ER was found to be closely associated with the phragmoplast and the forming cell plate (Hepler, 1982). More recently, the exclusion of plastids from the phragmoplast was described based on DIC images (Schopfer and Hepler, 1991). One study tried to compare the distribution of several organelles at the same time by employing a double-staining approach that allowed the simultaneous identification of Golgi stacks, mitochondria, and plastids in thin sections of embedded material under the fluorescence light mi-

croscope (Kawazu et al., 1995). In this case it was found that Golgi stacks approach the maturing cell plate more closely than mitochondria and plastids (Kawazu et al., 1995). However, the segregation of organelles during metaphase described here was not recognized in the earlier study, presumably due to the thinness of the sections (0.5  $\mu\text{m}$ ), which did not allow an appreciation of the three-dimensional arrangement of organelles over larger volumes.

#### Maintenance of Organelle Segregation Is Only Partially Dependent on Intact Cytoskeletal Elements

The segregation of Golgi stacks, mitochondria, and plastids into distinct domains demonstrates that plant cells are able to specifically control the positioning of these organelles during mitosis, but not necessarily during interphase (Fig. 7). We tested whether MFs or MTs are required for the setup and maintenance of the observed segregation by selectively disrupting either of the cytoskeletal elements by specific drugs. However, the treated cells did not progress through mitosis or cytokinesis, thus precluding us from observing any drug effects on organelle rearrangements. In a similar manner, we were not able to follow cells as they created the initial segregation of organelles. Thus we can only assess the role of the cytoskeleton in maintaining the previously setup organelle distributions. Both MF- and MT-disrupting drugs had only limited effect on organelle segregation in cells maintained in a perfusion chamber (Fig. 10B and data not shown), suggesting that these cytoskeletal arrays play only a minor role in keeping the organelles segregated. A complete collapse of the spindle by propyzamide treatment during continuous agitation resulted in a more profound effect on organelle distribution, although clusters of Golgi stacks were still maintained (Fig. 10C). This finding indicates that the spindle plays a central role in organizing the cytoplasm during metaphase. At the same time, the persisting Golgi clusters suggest that the stacks may be bound to other cellular structures, allowing them to maintain their association even after removal of the major organizing principle.

It is well known that the tight packing of spindle MTs excludes all larger organelles and therefore leads to an accumulation of these organelles in the surrounding phragmosome. However, the striking segregation of Golgi stacks and mitochondria plus plastids goes beyond this simple steric exclusion and appears to be at least partially dependent on the presence of an intact mitotic spindle. The role of the spindle MTs in organizing this segregation of organelles can be either direct or indirect. A direct role could be envisioned as a physical interaction between a Golgi-associated MT-binding protein and the spindle MTs, which results in the specific localization of Golgi stacks. The indirect model would postulate

that the spindle serves as an anchor for a separate signal that can recruit Golgi stacks into this area. Our data do not allow us to distinguish between these possibilities.

A central implication of the observed sorting of Golgi stacks is that the organelles and/or their target regions in the phragmosome are distinguished by specific molecules that facilitate the sorting process. A possible candidate molecule for the sorting might be myosin. Indeed it has been shown for pollen tubes that different types of myosin are associated with organelles of different sizes (Miller et al., 1995). We have previously demonstrated that Golgi stacks employ myosin motors for movements along MFs during interphase, although such rapid movements were not visible during mitosis and cytokinesis (see Mineyuki et al., 1984). The presence of actin filaments near Golgi stacks in dividing cells (Fig. 11 and data not shown) is also consistent with an acto-myosin based localization process. The more limited distribution of Golgi stacks compared with that of MFs may be caused by specifically localized "stop signals" (Nebenführ et al., 1999) that prevent the Golgi stacks from leaving these areas. A possible candidate for such a stop signal is calcium, since elevated levels of this ion are known to inhibit myosin motors in plants (Shimmen and Yokota, 1994; Yokota et al., 1999). A likely source of calcium is the ER at the spindle poles, which contains high concentrations of calcium (Wolniak et al., 1980). In this context the possible role of the spindle MTs may be the localization of ER to the spindle poles (Fig. 3B; Hepler, 1980). However, other areas of Golgi stack accumulation (Golgi belt, phragmoplast) lack such conspicuous calcium stores (Wolniak et al., 1980). Clearly more research is necessary to clarify the mechanisms of Golgi stack recruitment and organelle segregation.

#### The Mitotic Spindle Plays Only a Minor Role in the Inheritance of Golgi Stacks

Although Golgi stacks play an important secretory role during cytokinesis in plant cells, they also have to be evenly partitioned to ensure that both daughter cells receive a functioning secretory system. The problem of Golgi inheritance has attracted much attention in mammalian cell culture cells where the Golgi apparatus is organized in to a single perinuclear complex, the Golgi ribbon. During mitosis, the Golgi ribbon breaks apart into smaller fragments, which are subsequently partitioned into the daughter cells (for review, see Roth, 1999). Association of a subset of these Golgi fragments with the mitotic spindle is thought to ensure near equal distribution of Golgi membranes (Shima et al., 1997, 1998).

In this study we describe a similar association of plant Golgi stacks with the mitotic spindle (Figs. 2, 7, and 8A), with almost identical numbers of stacks present at both poles ( $50\% \pm 1\%$ ). Nevertheless, the

numbers of stacks differed considerably between sister cells and appeared to depend on their relative cell sizes. Normalization of Golgi stack numbers to the approximate cytoplasmic volumes of the two cells accordingly showed smaller deviations in Golgi stack densities. Thus it appears that the random cytoplasmic distribution Golgi stacks at metaphase likely plays a more important role in Golgi stack inheritance than the ordered arrangement of stacks around the spindle. This conclusion is further supported by the small proportion of stacks in the immediate vicinity of the spindle (Table I).

It has been reported that a doubling of the number of Golgi stacks occurs during metaphase (Garcia-Herdugo et al., 1988). We have tried to document this event in living BY-2 cells transformed with our Golgi marker. However, we were not able to identify any specific time period during which the number of Golgi stacks showed such a dramatic increase (data not shown). This failure to observe Golgi doubling may have resulted from the large number of stacks present in BY-2 cells (often more than 1,000) and the dynamic distribution of these stacks, which made it effectively impossible to account for all stacks present in a cell. Alternatively, the doubling of Golgi numbers in BY-2 suspension cultures may not occur within such a narrow time window as documented for root-tip cells (Garcia-Herdugo et al., 1988). Our observation that larger interphase cells contain more Golgi stacks (not shown) is also consistent with this interpretation.

#### Golgi Stacks Accumulate at Specific Sites to Ensure Rapid Formation of the Cell Plate

The observed segregation of Golgi stacks, mitochondria, and plastids also raises the question whether these organelles perform specific functions at their respective positions, or whether specific locations serve just as "parking" sites during mitosis and cytokinesis. In other words, are any of these organelles recruited to specific places within the phragmosome to satisfy a functional need at this location? For Golgi stacks near the phragmoplast, this possibility is most likely true. The role of Golgi-derived vesicles in cell plate formation has been documented extensively (Staehelein and Hepler, 1996; Heese et al., 1998) and an accumulation of Golgi stacks near the growing edge of the cell plate makes good biological sense. This observation therefore lends support to the concept that Golgi stacks can be recruited to places where their products are needed (Nebenführ et al., 1999).

A similar argument can be made for the Golgi stacks that accumulate near the spindle poles or in the Golgi belt. For example, the stacks in the Golgi belt may be involved in preparing the plasma membrane and cell wall of the future division site for the anticipated fusion of the cell plate (Mineyuki and

Gunning, 1990). In a similar manner, the Golgi stacks accumulating near the spindle poles may already be involved in the production of vesicles destined for the cell plate. Such a population of "prefabricated" cell plate components could help explain the remarkable rate of initial cell plate growth (20  $\mu\text{m}$  in diameter within a few minutes of cell plate initiation) despite the limited number of Golgi stacks in the region between the reforming nuclei and the phragmoplast (Figs. 5 and 9D; see Gunning, 1982). Experiments with the drug brefeldin A, which inhibits secretion and leads to a breakdown of the Golgi apparatus have demonstrated that the early cell plate is formed from material that has left the Golgi in metaphase, long before cell plate formation starts (Yasuhara et al., 1995). These results therefore support the idea that Golgi stacks at the spindle poles are already actively producing cell plate material.

### Is the Transport between ER and Golgi Altered during Mitosis and Cytokinesis?

During our observations of cells undergoing mitosis or cytokinesis we noticed that the green fluorescent label, which during interphase largely is confined to Golgi stacks (Nebenführ et al., 1999), also appeared as a hazy staining particularly in the mitotic spindle and the phragmoplast. Comparison of this pattern with cells expressing an ER-targeted GFP (GFP-hdel) suggests that this diffuse fluorescence represents the ER (Fig. 3). The apparent increase in GmMan1-GFP fluorescence in the ER during mitosis could result from one of several changes in the endomembrane system. For example, the ER of cells undergoing mitosis and cytokinesis redistributes dramatically from the cortical cytoplasm into the phragmosome, leading to a concentration of this organelle in the spindle and the phragmoplast (Figs. 3B and 4; see Hepler, 1980, 1982; Schopfer and Hepler, 1991). Thus the small amount of GmMan1-GFP, which is present in the ER throughout the cell cycle may be sufficient to appear as hazy fluorescence in regions of high ER density. This interpretation does not invoke any changes in membrane transport and relies simply on the redistribution of ER together with a relatively small number of (at steady state) ER-localized GmMan1-GFP molecules.

Alternatively, the apparent increase in ER fluorescence could result from a reduction of anterograde ER-to-Golgi transport of newly synthesized protein, or from an increased retrograde Golgi-to-ER transport of already existing protein, or both. A reduced export from the ER would be consistent with earlier reports suggesting a change in membrane flux through the secretory system (e.g. Mollenhauer and Mollenhauer, 1978). We have tried to quantify the relative contribution of Golgi and ER to total cell fluorescence at different stages of the cell cycle. It is unfortunate, though, that the values derived from

this analysis are inherently unreliable since we were not able to unequivocally distinguish between the signals from the two organelles due to the intermingling of ER and Golgi stacks in large parts of the cells. We are therefore not able to address the relative contribution of this potential change in ER-Golgi transport to the observed fluorescence pattern.

### CONCLUSIONS

We have determined the distribution of several organelles, namely Golgi stacks, ER, mitochondria, and plastids, in living, dividing plant cells. We show for the first time that during metaphase these organelles are sorted to distinct cytoplasmic regions. This segregation of organelles is largely maintained throughout cytokinesis. The accumulation of Golgi stacks near the spindle poles and in the Golgi belt, the future site of cell division, suggests specific roles for these stacks during cell plate formation and is therefore consistent with our model predicting Golgi stack recruitment to sites where their products are needed (Nebenführ et al., 1999).

### MATERIALS AND METHODS

#### Cell Lines and Culture Conditions

The transgenic BY-2 cell line expressing a fusion of GmMan1 and GFP (GmMan1-GFP) has been described previously (Nebenführ et al., 1999). BY-2 cells expressing GFP with an ER-retention signal (GFP-hdel) were kindly provided by M. Ziegler and K. Danna (University of Colorado, Boulder). Cells were grown in a modified Linsmaier and Skoog medium (Nagata et al., 1982) with constant shaking (120 rpm) at 27°C in the dark. Cells were subcultured weekly into fresh medium at a dilution of 1:50. Cells were harvested by low-speed centrifugation (500g for 2 min) 2 to 4 d after subculturing and immediately used for experiments. Treatment of the cells with anticytoskeletal drugs was performed as described (Nebenführ et al., 1999).

#### Microscopy

Mitochondria and plastids were fluorescently labeled with 50 nM MitoTracker Red CMXRos (Molecular Probes, Eugene, OR) for at least 15 min in the dark prior to observation. Cells were observed on an epifluorescence microscope (DM-RXA, Leica, Wetzlar, Germany) with standard fluorescein isothiocyanate and Cy3 filter sets. Extended observations of living cells were carried out in a perfusion chamber with a continuous supply of fresh medium at a flow rate of 0.5 mL/min. No additional MitoTracker dye was added during observations in the perfusion chamber. Images were captured with a Sensicam (Cooke Corp., Tonawanda, NY) and SlideBook software (Intelligent Imaging Innovations, Denver, CO) on a Macintosh computer (Apple Computer, Cupertino CA). Image processing occurred in SlideBook (deconvolution), NIH Image (three-dimensional reconstructions, quantifications; program avail-

able at <http://rsb.info.nih.gov/nih-image/Default.html>), and Photoshop (final image assembly; Adobe Systems, San Jose, CA). Stack distributions were quantified in NIH Image. Positions of individual stacks were either marked by hand or automatically using a peak-finding algorithm followed by manual corrections. Regions of interest (entire cell, Golgi belt, perinuclear cortex, spindle region, and cell interior) were selected manually and the number of stacks within them determined using the "Analyze Particles" command. Duplicate stacks that were marked in adjacent slices of three-dimensional images were subtracted from the total. Cytoplasmic volume was estimated by measuring the area of combined GFP and MitoTracker fluorescence after background subtraction (which largely removes the ER-like fluorescence) in a three-dimensional-image series. All values given in the text are arithmetic means  $\pm$  SE.

### Immunofluorescence

BY-2 cells 2 to 4 d after subculturing were fixed with 1% (v/v) glutaraldehyde in culture medium for 15 min. After two washes in phosphate-buffered saline (PBS), the cells were transferred to 4 to 7 mL of PBS containing 0.5% (w/v) NaBH<sub>4</sub> to reduce autofluorescence of the fixative. After this overnight incubation, cells were again washed in PBS and their cell walls were partially digested by treatment with 1% (v/v) cellulase (Worthington, Freehold, NJ) and 1% (v/v) pectinase (Fluka, Buchs, Switzerland) for 30 min. Following two additional washes in PBS, the cells were allowed to settle onto a multiwell microscope slide coated with 0.1% (w/v) polyethyleneimine. Membranes were permeabilized by a 15-min treatment with 1% (w/v) IGEPAL (Sigma, St. Louis). To detect MTs, a mouse monoclonal antibody against *Drosophila melanogaster*  $\alpha$ -tubulin was used (1:30; M. Fuller, Stanford University, Stanford, CA). Actin MFs were detected with a mouse monoclonal antibody against pea actin (undiluted; Andersland et al., 1994). The secondary antibody for both antitubulin and anti-actin antibodies was anti-mouse Alexa594-IgG (1:200; Molecular Probes). Primary and secondary antibody incubations lasted for at least 1 h each and were followed by at least three 5-min washes in PBS. The first wash after the secondary antibody application included 0.1  $\mu$ g/mL 4',6-diamino-phenylindole (Molecular Probes) to stain DNA. After the final wash the cells were either mounted in 50% (w/v) glycerol in PBS or viewed directly.

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