Comment

W(h)ither the Golgi during Mitosis?

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During mitosis, the entire content of the cell must be divided equally between two daughter cells before karyo- and cytokinesis are completed. How is this complex process accomplished? In the case of the Golgi, it was found many years ago in tissue culture cells that the characteristic stack-like organization of the Golgi fragmented at the onset of mitosis (Robbins and Gonatas, 1964; Lucocq and Warren, 1987), and that Golgi fragments were subsequently distributed to each daughter cell. The identity of these Golgi fragments and the mechanisms involved in Golgi fragmentation and in the segregation of membranes and Golgi contents to daughter cells are currently the subject of considerable study and debate.

In this issue of *JCB*, three papers are published on Golgi fragmentation during mitosis (Kano et al., 2000; Lowe et al., 2000; Colanzi et al., 2000), which, together with papers published recently in *Cell* (Zaal et al., 1999) and *Molecular Biology of the Cell* (Terasaki, 2000), provide new insights into Golgi fragmentation, the mechanisms involved, and the experimental difficulties in analyzing this process. The background of these papers is also discussed, however, this commentary is not an exhaustive review of the literature on Golgi inheritance during mitosis (see Warren and Wickner, 1996). Nor is it a treatise on different models of Golgi inheritance or the pros and cons for those models. It is written from the perspective of an outsider, like most of you reading this, who wants to understand from the printed word and published data what happens to the Golgi during mitosis.

We will consider two points. First, changes in Golgi organization during mitosis: we will discuss evidence for different stages in Golgi fragmentation, whether there is retrograde membrane flow from the Golgi to the ER, and the possible significance of the ER as an end-point in this process. Second, mechanisms underlying these changes: we will discuss the evidence for roles of MEK1 (mitogen-activated protein kinase kinase, MAPKK)[1] and Cdc2, whether these kinases work alone or sequentially in Golgi fragmen-

tation, and consider the evidence for specific targets of these kinases that might mediate Golgi fragmentation.

Changes in Golgi Morphology during Mitosis: Stacks, Blobs, and ER

Stages in Golgi Fragmentation

In this issue of *JCB*, Kano et al. (2000) describe changes in Golgi organization during mitosis by examining MDCK epithelial cells expressing the Golgi enzyme galactosyltransferase tagged with green fluorescent protein (GalT-GFP). To induce mitotic-specific changes in the Golgi, Kano et al. (2000) perforated the cell membrane with the bacterial toxin streptolysin O, washed the cells with 1 M KCl to remove cytosolic and peripheral membrane proteins, and then added back cytosol from metaphase-arrested *Xenopus* eggs and an ATP regenerating system. Confocal microscopy revealed a rapid change in Golgi morphology from a perinuclear, reticular organization to a more fragmented structure composed of large blobs, the number of which appears to diminish over time. During this time there is also an increase in diffuse background fluorescence throughout the cell and the appearance of GalT-GFP fluorescence in the nuclear envelope. This is shown in Figure 3 B of Kano et al. (2000), in which a flat cell in low density culture is examined. (Figures 1, 2, and 3 A of Kano et al. [2000] examined confluent monolayers of cells in which the height and narrowness of the cell compromise the interpretation of the data, and laser damage from continuous confocal imaging over long periods was deleterious.) These fragmented Golgi structures are investigated by electron microscopy (see Figure 4; immunoelectron microscopy was not performed to identify membranes), and by immunofluorescence microscopy to compare the distribution of GalT-GFP with that of an ER marker protein (protein disulfide isomerase, PDI; see Figure 5 B). The immunofluorescence data show colocalization of GalT-GFP and PDI in the nuclear envelope (mid-section) and peripheral membranes in the apical confocal section of the cell, although larger GalT-GFP blobs in this section are clearly not labeled with PDI. Kano et al. (2000) conclude that the Golgi changes from a stack-like reticulum to a more fragmented organization, composed of large membranous blobs that have a Golgi identity (based on the absence of an ER marker) and a membrane with an ER identity, which has a more diffuse distribution throughout the cytoplasm.

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[1]*Abbreviations used in this paper:* ERK, extracellular signal-regulated protein kinase; GalT-GFP, galactosyltransferase tagged with green fluorescent protein; ManII-GFP, mannosidase II-GFP; MEK1, mitogen-activated protein kinase kinase, MAPKK; NRK, normal rat kidney; PDI, protein disulfide isomerase.

Is the ER an End-Point in Golgi Fragmentation?

The description by Kano et al. (2000) of changes in Golgi organization induced by mitotic cytosol supports recent observations by Zaal et al. (1999) and Terasaki (2000). Zaal et al. (1999) examined GalT-GFP distribution in intact HeLa, CHO, and PTK1 cells, rather than permeabilized cells. They report that the steady-state ratio of GalT-GFP between the Golgi and ER is $\sim$70:30 (see Figure 1 of Zaal et al., 1999). Furthermore, they suggest that this ratio reflects a steady-state distribution of GalT-GFP due to cycling of the protein between these membrane compartments. This suggestion is supported by several independent experiments in which GalT-GFP accumulates in the ER when the Golgi is dispersed (nocodazole; see Figure 3), or when ER export but not retrograde Golgi to ER transport is blocked (expression of a dominant-negative Sar1p; see Figure 3), or when the ER pool of GalT-GFP is removed by photobleaching (see Figure 2).

Zaal et al. (1999) analyzed the distribution of GalT-GFP in cells entering mitosis. They observe several, apparently sequential stages in Golgi reorganization, beginning with the loss of the perinuclear reticular structure, the appearance of numerous, small non-motile structures (similar to those described by Kano et al., 2000), which grew fainter and disappeared, and finally a diffuse fluorescence throughout the cytoplasm (see Figure 4 of Zaal et al., 1999). Zaal et al. (1999) note that small, GalT-GFP positive structures are observed occasionally, but the diffuse staining pattern predominates (>98% of the fluorescent signal). Similar, diffuse distributions of other Golgi marker proteins, including endogenous GM130 (see below), were observed (see Figure 4 B). Immunoelectron microscopy of mannosidase II-GFP (ManII-GFP) and endogenous GalT revealed that in mitotic cells these Golgi resident proteins are present in tubuloreticular structures coated with ribosomes (see Figure 5), indicating an ER location. Zaal et al. (1999) examined the mobility of GalT-GFP and lipids in interphase and mitotic cell peripheral membranes (ER) using photobleaching. Results show that both diffuse rapidly and neither have a substantial immobile fraction, which the authors interpret as evidence that GalT-GFP is in a continuous membrane system (e.g., ER) rather than a population of vesicles (but see Jesch and Linstedt, 1998 for evidence from subcellular fractionation studies that in mitosis a portion of Golgi residents are in vesicles).

Zaal et al. (1999) propose that reorganization of the Golgi during mitosis reflects the reversion to a constitutive pathway of membrane cycling between the Golgi and the ER, in which the rate of retrograde cycling from the Golgi to ER is greatly increased over the rate in interphase cells (see also Terasaki, 2000). In this view, Golgi fragmentation induced by brefeldin A, nocodazole or okadaic acid (see below) reflects the accumulation of Golgi membranes and contents at one (intermediate) stage or another of a constitutive cycle in which complete absorption of the Golgi membranes into the ER is the extreme conclusion. A counter argument is that these stages represent divergent pathways that do not reflect the normal process of Golgi fragmentation during mitosis (Lowe et al., 2000; see below).

Are Golgi Blobs an Intermediate or an End-Point in Golgi Fragmentation?

Terasaki (2000) examined Golgi dynamics (marked with KDELR$_m$-GFP) in sea urchin embryos during successive cleavages of blastomeres. He also observed the formation of numerous Golgi blobs during the onset of mitosis, and that the number of these blobs decreased rapidly during mitosis, although a corresponding increase in (diffuse) cytoplasmic staining was not apparent, perhaps due to the shape of the cells. Previous studies by Shima et al. (1998) used a GFP chimera of N-acetylglucosaminyl-transferase (NAGFP) to examine Golgi morphology in mitosis in HeLa and VERO cells, and found that the Golgi stack reorganized into numerous bright blobs in the cytoplasm, which decreased in size during mitosis. However, blobs containing NAGFP are quite numerous in the cytoplasm during metaphase, although it is clear from the images that a diffuse background fluorescence signal also increases during mitosis (see Figure 3 of Lowe et al., 2000). In their most recent study in this issue of *JCB*, Lowe et al. (2000) show confocal images of GM130 staining in metaphase normal rat kidney (NRK) and HeLa cells which reveal numerous GM130-positive blobs (see Figure 2 D), whereas Zaal et al. (1999) showed, with the same antibody, a uniform, diffuse distribution of GM130 with no blobs (see Figure 4 B of Zaal et al., 1999). Lowe et al. (2000) quantify the amount of the Golgi marker protein GM130 in these Golgi fragments (blobs) in mitotic cells and report that after subtraction of background staining they find that $\sim$50% of GM130 is present in the blobs (see Table I of Lowe et al., 2000; the location of the other 50% of GM130 staining is not defined). In contrast, Zaal et al. (1999) reported that 2% of GalT-GFP is in blobs, and the remainder is diffusely distributed in the cytoplasm (ER). Whether this difference in distributions of GM130 and other Golgi residents reflects the method of preparing cells for immunofluorescence (Lowe et al., 2000 used 100% methanol to fix/permeabilize cells, whereas Zaal et al., 1999 used 2% formaldehyde followed by antibody in PBS containing 0.15% saponin) is unclear.

Implications of a Golgi–ER Connection for Golgi Reassembly

The presence of GalT-GFP in the ER during mitosis may reflect the accumulation of newly-synthesized protein. Zaal et al. (1999) showed that in the presence of the protein synthesis inhibitor cycloheximide, GalT-GFP still appeared in the ER in interphase cells presumably as a result of retrograde transport from the Golgi (Figure 2 of Zaal et al., 1999), but this experiment was not shown for mitotic cells. However, Farmaki et al. (1999), who also found GalT in ER membranes (and Golgi blobs) in mitotic cells, showed that cycloheximide significantly reduced the amount of GalT in the ER of mitotic cells (Figure 9 of Farmaki et al., 1999).

If (a portion of the) Golgi resident proteins localize to the ER during mitosis, then reformation of the Golgi could involve export of proteins and membrane from the ER leading reassembly of the Golgi stack. A reasonable test of this hypothesis is to use a dominant-negative mutant of Sar1p (mSar1p) to block ER export of proteins at

the end of mitosis and examine the effects on Golgi reassembly. Zaal et al. (1999) micro-injected mSar1p into an asynchronous cell population, then fixed cells 4 h later and looked for cells that had completed mitosis (decondensing chromatin in irregularly shaped nuclei in adjacent cells). The data in Figure 8 of Zaal et al. (1999) show two apparent daughters cells in which GalT-GFP is diffusely distributed throughout the cytoplasm, although a few bright blobs of fluorescence are apparent in one of the daughter cells. Zaal et al. (1999) conclude that the Golgi did not reassemble because export of Golgi resident proteins form the ER was inhibited. Shima et al. (1998) micro-injected purified mSar1p into cells at the G2/M boundary in a population that had been synchronized at G1. Their results show that NAGFP accumulates in blobs throughout the cytoplasm during pro-metaphase and metaphase (Figure 2, a–d of Shima et al., 1998). Shima et al. (1998) conclude that Golgi fragmentation does not involve dissolution of a Golgi resident protein into the ER. Although these results appear to be contradictory, it is clear that these experiments assay a different end-point in Golgi reorganization. Zaal et al. (1999) examined whether the Golgi reassembles following mitosis when protein export from the ER is blocked by mSar1p. In contrast, Shima et al. (1998) examined whether the Golgi undergoes initial fragmentation in mitotic cells expressing mSar1p; cells were not examined through to cytokinesis to determine whether Golgi stacks reassemble in daughter cells expressing mSar1p.

Linking Golgi Stacks–Blobs–ER: A Compromise Pathway for Golgi Fragmentation

Is there a consensus description of changes in Golgi organization during mitosis? All of the studies cited above describe an initial change in Golgi morphology from a perinuclear, reticular stack-like structure in interphase, to a less organized structure of large blobs distributed throughout the cytoplasm in early stages of mitosis. At this point, there appears to be a divergence in opinion. At one extreme, it seems that >98% of these Golgi blobs disappear, and Golgi resident proteins appear in tubulovesicular membranes containing ER proteins and ribosomes. At the other extreme, the size of Golgi blobs may diminish but many of the blobs remain intact (comprising ~50% of a Golgi resident). In between these extremes, some Golgi resident proteins appear in a diffuse, cytoplasmic membrane distribution with ER markers, whereas another population is localized to Golgi blobs. That there is some variance in Golgi reorganization during mitosis is perhaps not surprising given that many cells can apparently accomplish segregation of Golgi stacks between daughter cells without the requirement for fragmentation (Stanley et al., 1997). Its seems feasible, therefore, that under some conditions (of reconstitution, see below) Golgi fragmentation may be partial (blobs), and that under other conditions it may result in complete dissolution of the Golgi into the ER.

Mechanisms of Golgi Fragmentation during Mitosis

From these descriptions of Golgi organization in mitosis we now turn to the mechanisms underlying these changes. Entry into mitosis is regulated by the activation of a number of kinases. Recent studies have reported that mitogen-activated protein kinase kinase (MAPKK, referred to here as MEK1) activity is required for G2/M transition, activation of Cdc2 and progression into mitosis (see Wright et al., 1999). Cdc2 activity is required for chromosome condensation, breakdown of the nuclear envelope, and assembly of the mitotic spindle (see Nigg, 1995). The roles of both MEK1 and Cdc2 in Golgi fragmentation have been examined.

Experimental Approaches

Two basic experimental approaches have been taken to dissect mechanisms of Golgi fragmentation. One involves methods similar to those employed by Kano et al. (2000), in which cells are permeabilized gently with detergent (e.g., digitonin), washed with 1M KCl to remove endogenous cytosol and peripheral membrane proteins, and then the cells are incubated in cytosol from mitotic (or interphase) cells together with an ATP regenerating system. Cytosol can be supplemented with purified proteins, inhibitors, etc., or depleted of proteins by affinity chromatography. Cells can then be processed directly for immunofluorescence or electron microscopy, or for biochemical analysis of proteins. The second method involves the use of Golgi-enriched membrane fractions from cells to which mitotic or interphase cytosol is added as above. After incubation, membranes are separated from cytosol by centrifugation through a sucrose pad. Membranes can then be processed for biochemical analysis of proteins. Experiments described below will be generally referred to as using cells or Golgi-enriched membranes, respectively, to describe these two methods.

A Role for MEK1 in Golgi Fragmentation

Initial studies of MEK1 (Acharya et al., 1998; Lowe et al., 1998) tested the effects of a specific inhibitor of MEK1, PD 098059, on Golgi fragmentation in NRK cells. Acharya et al. (1998) showed a concentration-dependent inhibition by PD098059 of both MEK1 activity and Golgi fragmentation (Figure 5 of Acharya et al., 1998), and that immune-depletion of MEK1 from mitotic cytosol reduced by 50% the activity of mitotic cytosol to induce Golgi fragmentation in permeabilized NRK cells (Figure 6). Lowe et al. (1998) showed an example of a nonpermeabilized mitotic NRK cell from a synchronized population treated with PD098059 in which the Golgi is clearly fragmented. Although this appears to contradict the observations by Acharya et al. (1998), other studies have shown that PD098059 blocks cells at the G2 boundary (see Wright et al., 1999); it is unclear, therefore, how cells in the study by Lowe et al. (1998) entered mitosis in the presence of this inhibitor. Using Golgi-enriched membranes, Lowe et al. (1998) also reported that although addition of PD098059 inhibited MEK1 activity in a dose-dependent manner, there was no effect on Golgi fragmentation, nor was there an effect when MEK1 protein was immune-depleted from mitotic extracts (Figure 6 of Lowe et al., 1998). Furthermore, addition of PD098059 to nonpermeabilized NRK cells blocked phosphorylation of two known downstream

targets of MEK1, ERK1 (extracellular signal-regulated protein kinase 1), and ERK2 (Figure 7). Acharya et al. (1998) also examined whether ERK1 and ERK2 were involved in MEK1-induced Golgi fragmentation in their assay by immune-depleting ERK2 from mitotic extracts (Figure 7 of Acharya et al., 1998), and by adding inactivated ERK2 to mitotic extract (Figure 8). Acharya et al. (1998) reported that depletion of ERK2 had no effect on the activity of mitotic extract to induce Golgi fragmentation, and that addition of an inactivated ERK2 caused a dose-dependent inhibition of Golgi fragmentation; presumably, the excess ERK2 competed MEK1 binding/activity away from other substrates that induce Golgi fragmentation. Acharya et al. (1998) conclude that MEK1 must be targeting substrates other than ERK2, and possibly ERK1, that are involved in Golgi fragmentation.

The conditions used to examine the effects of PD098059 were different in these experiments, which could explain the different results: Acharya et al. (1998) used cells permeabilized with digitonin, washed with 1M KCL, and then reconstituted with mitotic extract for 1 h; Lowe et al. (1998) used Golgi-enriched membranes reconstituted with mitotic extract for 30 min. A possible resolution of these differences is that the Golgi-enriched membranes used by Lowe et al. (1998) may have been stripped of the MEK1 target during isolation, and thereby Golgi fragmentation would be refractory to the MEK1 inhibitor, PD098059; presumably, the target(s) are not removed by the 1-M KCl wash applied to permeabilized cells (Acharya et al., 1998).

Upstream and Downstream Components of a MEK1 Pathway

In this issue of *JCB*, Colanzi et al. (2000) extend the analysis of MEK1 in Golgi fragmentation. They report that MEK1 is hyperphosphorylated in mitotic extract, although the site(s) of phosphorylation on MEK1 and the upstream kinase(s) and downstream target(s) involved are as yet unknown (see Figures 1 and 2 of Colanzi et al., 2000). Removal of eight NH_2-terminal amino acids by bacterial anthrax toxin is known to block MEK1 binding to ERK1 and ERK2. Cleavage of this domain of MEK1 did not inhibit hyperphosphorylation of MEK1 by mitotic extract, or induction of Golgi fragmentation in NRK cells (see Figures 4 and 5). This confirms a previous result from Lowe et al. (1998) who showed with Golgi-enriched membranes that bacterial anthrax toxin inhibited ERK1 and ERK2 phosphorylation (by MEK1) and mitotic cytosol treated with anthrax toxin induced Golgi fragmentation (Figure 7). Although Lowe et al. (1998) interpret this result to mean that MEK1 is not involved in Golgi fragmentation, Colanzi et al. (2000) conclude that phosphorylation of MEK1 by an unknown kinase in mitotic extract promotes MEK1 activity towards a substrate(s), other than ERK1/2, that is involved in Golgi fragmentation (see also Acharya et al., 1998, and above). A good test of mitotic activation of MEK1 by an upstream kinase would be to use bacterially expressed, nonphosphorylated MEK1, which was shown previously to rescue the Golgi fragmentation activity when added to MEK1-depleted mitotic extract (Acharya et al., 1998), and determine whether it is specifically hyperphosphorylated by mitotic extract. Clearly, identifi-

cation of putative MEK1 target(s) that are not ERK1 or ERK2 will be critical in clearing up remaining ambiguities in these experiments and results, and for elucidating mechanisms involved in Golgi fragmentation by MEK1.

A Role for Cdc2 in Golgi Fragmentation

Although MEK1 target(s) involved in Golgi fragmentation are yet to be uncovered, potential targets for Cdc2 have been examined in detail. Previous studies described a series of protein-protein interactions in the Golgi involving a putative structural Golgi matrix protein GM130, that binds to both a Golgi cisternal membrane-associated protein GRASP65, and to a vesicle protein p115, which in turn binds to a Golgi vesicle membrane protein, giantin (Nakamura et al., 1997). During Golgi fragmentation at the onset of mitosis, these protein-protein interactions are disrupted.

Lowe et al. (1998) examined whether any of these proteins are a target of Cdc2 kinase activity. They showed that GM130 on Golgi-enriched membranes is phosphorylated upon addition of mitotic extract or purified Cdc2, and that depletion of Cdc2 from mitotic extract significantly reduced GM130 phosphorylation (Figure 2 of Lowe et al., 1998) and Golgi fragmentation (Figure 5). Whether GM130 on Golgi membranes is directly phosphorylated by Cdc2 kinase, or Cdc2 activates a downstream kinase that phosphorylates GM130, or whether there are additional targets of the Cdc2 kinase cascade that cause Golgi fragmentation is unclear. That GM130 may be a direct substrate for Cdc2 is implicated by the presence of a putative Cdc2 phosphorylation site on Ser25 (Figure 3), and that NH_2-terminal peptide of GM130 can be phosphorylated in vitro by Cdc2 (Figure 2).

Seemingly complementary experiments were performed by Acharya et al. (1998) in which Golgi fragmentation was examined in permeabilized NRK cells incubated with mitotic extract that had been depleted of Cdc2, or with fractions of mitotic extract that had been separated by phosphocellulose chromatography (Figure 3 of Acharya et al., 1998). Acharya et al. (1998) report that Cdc2-depleted mitotic extract induces Golgi fragmentation, and that fractions enriched in Cdc2 from the phosphocellulose column do not induce Golgi fragmentation. Although these data appear to contradict the results of Lowe et al. (1998), it is reasonable to speculate that the different sources of Golgi may be responsible. Cdc2-independent Golgi fragmentation in permeabilized cells could be due to retention of some endogenous Cdc2 activity (but see Figure 6 in Colanzi et al., 2000), or the presence of another kinase(s) that can be activated in a Cdc2-independent manner by other factors in the mitotic extract. Cdc2-dependent Golgi fragmentation in isolated, Golgi-enriched membranes could be due to the loss (due to the isolation procedure) of kinases that could either substitute for Cdc2 or can be activated by other factors in mitotic extract. In both cases, one of these kinases may be MEK1. Nevertheless, the data from Lowe et al. (1998) show that GM130 is phosphorylated by mitotic extract and that Ser25 is a site for phosphorylation, both of which correlate with Golgi fragmentation, and that Cdc2 is involved directly or indirectly in this process. Whether MEK1 or Cdc2 is redundant in this

process, or acts sequentially will be discussed below when we return to the Kano et al. (2000) paper.

A Role for GM130 Phosphorylation and Dephosphorylation in Golgi Organization

In this issue of *JCB*, Lowe et al. (2000) continue the analysis of the role of GM130 phosphorylation in Golgi fragmentation in mitosis. They raise an antibody (P25) to a synthetic peptide comprising residues 21–31 of GM130 that includes phosphorylated serine25. Using this important reagent, Lowe et al. (2000) examined the expression and subcellular distribution of the mitotic-specific, phosphorylated form of GM130. As expected from their previous studies (see above), they find that P25 reacts with GM130 only during mitosis (Figure 4 C of Lowe et al., 2000) and stains predominantly large Golgi blobs throughout the cytoplasm of those cells (Figures 2–4). Little or no background staining is observed with P25, but the level of GM130 in these Golgi blobs is reported to be ~50% of total GM130 staining in the cells (Table I). If the remainder is background staining in the cytoplasm (ER?) then the lack of P25 staining there may reflect the presence of dephosphorylated GM130. A test of this notion would be to determine the percentage of total GM130 that is phosphorylated on ser25 during different stages of mitosis, and the subcellular distribution of GM130 that is not phosphorylated on ser25.

One consequence of ser25 phosphorylation of GM130 is that GM130 binds poorly to its partner p115 (see also Lowe et al., 1998). Double immunofluorescence of mitotic cells with P25 and p115 antibodies reveals some overlap in staining patterns (Figure 4 A of Lowe et al., 2000), but quantification of the apparent ratio of P25/p115 in Golgi blobs shows a decrease from 1 in interphase cells to ~0.22 in mitotic cells (Lowe et al., 2000; Table II). In contrast, Golgi fragmentation induced by ilimaquinone or nocodazole results in Golgi blobs that do not react with P25, but strongly costain with GM130 and p115 antibodies (Figure 5 C of Lowe et al., 2000). Again, two conclusions may be drawn from these results. First, drug-induced Golgi fragmentation involves pathways different from those used for Golgi fragmentation in mitosis that require GM130 phosphorylation and GM130/p115 dissociation. Second, Golgi fragmentation does not require GM130 phosphorylation and GM130/p115 dissociation per se, and that even in mitosis alternate, or parallel pathways are available to induce Golgi fragmentation (see below).

P25 staining of ser25 phosphorylated GM130 disappears upon completion of mitosis (Figures 2 and 4 C), indicating that GM130 becomes dephosphorylated. Therefore, Lowe et al. (2000) investigated whether phosphatases are involved in Golgi fragmentation, and in regulating the phosphorylation state of GM130. Addition of the protein phosphatase PP1 and PP2A inhibitor, okadaic acid, had little or no effect on mitotic extract-induced Golgi fragmentation in whole cells, but P25 staining was weak to absent and GM130 and p115 colocalized strongly on Golgi blobs as expected if GM130 is not phosphorylated on ser25 (Figure 5 A). Given the suggested role of cdc2 in Golgi fragmentation (see above), this result is somewhat surprising as okadaic acid has been reported previously to accelerate entry into mitosis by eliciting the premature hyperphosphorylation of cdc25 which can then dephosphorylate and activate cdc2 (Kumagai and Dunphy, 1992). However, Lowe et al. (2000) got a more expected result by performing the same experiment with Golgi-enriched membranes; in the presence of okadaic acid, GM130 was phosphorylated on ser25 (P25 reactive) and p115 was not associated with the membranes (Figure 5 B). Lowe et al. (2000) go on to show that addition of interphase cytosol results in loss of GM130 phosphorylation on ser25 in a dose-dependent and okadaic acid inhibited manner (Figure 6, A and B). The responsible phosphatase is shown to be PP2A, rather than PP1 using specific inhibitors (Figure 6 C), purified protein (Figure 7), and reconstituted PP2A complexes (Figure 8). Although cell fractionation shows that PP2A is enriched in membranes containing Golgi, it would be important to show Golgi localization by immunofluorescence microscopy. It will also be interesting to understand mechanistically how phosphorylation and dephosphorylation of GM130 leads to changes in p115 binding and Golgi fragmentation.

Partner Mechanism: Do MEK1 and Cdc2 Work Sequentially?

Clearly, kinases and phosphatases are involved in many aspects of Golgi fragmentation, and the question arises whether different kinases, for example, are redundant or act sequentially in facilitating Golgi inheritance during mitosis. We now return to the paper by Kano et al. (2000) published in this issue of *JCB*. Recall that Kano et al. (2000) provided evidence for Golgi fragmentation into blobs, and partial dissolution of Golgi into ER (see above). By staging the extent of Golgi fragmentation in cells as intact (no fragmentation), punctate (blobs) or dispersed (ER distribution), Kano et al. (2000) asked whether inhibitors of MEK1 and Cdc2 act in a stage-specific manner to block Golgi fragmentation induced by mitotic extract (Figures 8–10 of Kano et al., 2000); unfortunately, final concentrations of these inhibitors are not given and assays of kinase inhibition are not shown. They report that butyrolactone, an inhibitor of Cdc2, did not effect Golgi fragmentation into blobs (>80% of cells) but inhibited the appearance of GalT-GFP in the ER (<10% of cells, compared with >65% of cells in the control); the MEK1 inhibitor PD098059 increased to 40% the number of cells unable to undergo Golgi fragmentation (similar to that caused by the broad-range kinase inhibitor staurosporine); and, a combination of butyrolactone and PD098059 inhibited all Golgi fragmentation in >80% of cells (Figure 8). These general trends in the effects of inhibiting Cdc2 and MEK1 on Golgi fragmentation are complemented by a separate series of experiments in which either kinase is depleted from mitotic extract (Figure 9). These results confirm roles of both MEK1 and Cdc2 in Golgi fragmentation. Finally, Kano et al. (2000) investigated whether activation of MEK1 with STE11, and/or activation of Cdc2 with cyclin A in interphase cytosol causes Golgi fragmentation (Figure 10). Although MEK1 is active in interphase cytosol, inhibition by PD098059 blocks all Golgi fragmentation in >75% of cells. STE11 activates MEK1 in interphase cytosol and causes GalT-GFP dispersal into the ER

in >60% of cells, whereas activation of Cdc2 with cyclin A causes GalT-GFP accumulation equally in intact Golgi, Golgi blobs, and the ER. Significantly, sequential activation of MEK1 and then Cdc2 causes essentially all of the cells to disperse GalT-GFP into the ER, whereas sequential activation of Cdc2 and then MEK1 causes the equal accumulation of GalT-GFP in Golgi blobs and the ER.

In conclusion, the results presented in papers published in this issue of *JCB* and elsewhere have sought to investigate pathways of Golgi fragmentation, and roles of MEK1 and Cdc2. The results and their interpretation are sometimes controversial and/or ambiguous. Often, 2 experiments that seem to address the same question have different outcomes. Such discrepancies may be due to differences in experimental approaches, in experimental endpoints, or in the weight given to results of different experiments.

Mitotic Golgi fragmentation appears to be a linear set of reactions that converts stacks to membranous blobs that undergo further reorganization through tubulovesiculation and become continuous with the ER membrane. However, it should be noted that initiation, let alone completion of this reaction might not be required for partitioning of the Golgi into daughter cells, since many cells accomplish this process without Golgi fragmentation. MEK1 and Cdc2 may work sequentially to drive the reorganization of the Golgi stack into blobs (MEK1 dependent), and then from blobs into a more dispersed state throughout the cytoplasm (Cdc2 dependent) which, in the extreme, can lead to reabsorption of Golgi membranes into the ER. Cdc2 may also have a role in initial Golgi fragmentation as blobs are formed when only Cdc2 is activated, and inhibition of both MEK1 and Cdc2 inhibits Golgi fragmentation to an extent greater than that when only one of them is inhibited. It is possible that these Cdc2-induced blobs are different from those formed by MEK1 activation and other events that induce Golgi fragmentation (e.g., nocodazole, brefeldin A, okadaic acid, ilimaquinone), or, more likely, represent another, perhaps later, intermediate stage in Golgi fragmentation in the same linear pathway that involves MEK1. Presumably, different assay conditions (and different cells) have the capacity to drive Golgi fragmentation to specific intermediates in this reaction, and not necessarily to completion.

Clearly, the next steps are to define substrates for MEK1 and Cdc2, and proteins associated with Golgi membranes (besides GM130) that are the end-point targets of these activators and/or their substrates. Other proteins implicated in Golgi fragmentation, including coatamer (Misteli and Warren, 1994), and protein Gβγ and protein kinase D (Jamora et al., 1999), may need to be incorporated into these pathways. The goal remains to determine mechanistically how target proteins of these different activators are involved in Golgi organization, Golgi fragmentation during mitosis, and Golgi reassembly at the end of telophase.

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