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Dynamics of proteins in Golgi membranes: comparisons between mammalian and plant cells highlighted by photobleaching techniques

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Abstract. In less than a decade the green fluorescent protein (GFP) has become one of the most popular tools for cell biologists for the study of dynamic processes in vivo. GFP has revolutionised the scientific approach for the study of vital organelles, such as the Golgi apparatus. As Golgi proteins can be tagged with GFP, in most cases without altering their targeting and function, it is a great substitute to conventional dyes used in the past to highlight this compartment. In this review, we cover the application of GFP and its spectral derivatives in the study of Golgi dynamics in mammalian and plant cells. In particular, we focus on the technique of selective photobleaching known as fluorescence recovery after photobleaching, which has successfully shed light on essential differences in the biology of the Golgi apparatus in mammalian and plant cells.

Key words. Green fluorescent protein; confocal microscopy; photobleaching; intracellular trafficking; Golgi dynamics.

Introduction to Golgi organisation – comparison between animal and plant cells

Mammalian cells

In both mammalian and plant cells, the Golgi apparatus has a central role in the modification and sorting of proteins, lipids and carbohydrates arriving from the endoplasmic reticulum (ER), and their subsequent transport to other cellular compartments. Despite an equally vital role of the Golgi in both animal and plant kingdoms, the organisation of this organelle is very different in the two biological systems.

The mammalian Golgi occupies a central position in the cell, over the microtubule organising centre (MTOC) in the perinuclear region (fig. 1 A). Protein transport from the ER to the Golgi occurs via ER exit sites, also known

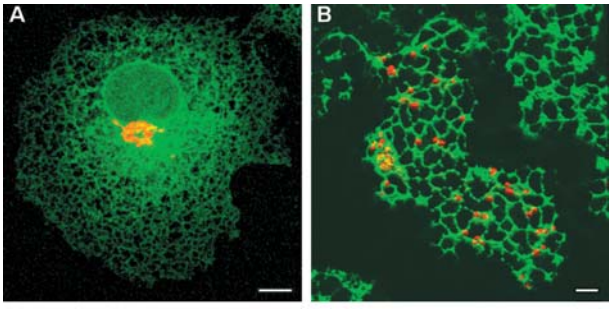

Figure 1. Comparative structure of Golgi apparatus in mammalian and plant cells. (*A*) The mammalian Golgi apparatus labelled with GalT-YFP (red) with the ER labelled with SRβ-CFP (green). Constructs were expressed in COS cells and imaged at 37 °C using confocal fluorescence microscopy. Note the compact perinuclear localisation of the Golgi while the ER reticulum is found throughout the cell. (*B*) Tobacco leaf epidermal cells transformed with ST-YFP (red) and ss-GFP (green), a form of GFP directed into the secretory pathway with a signal sequence but retained in the ER by an HDEL peptide. Note the ER network closely associated with multiple Golgi bodies found throughout the cell. The image is the result of a 3D reconstruction and rendering by Velocity software. Scale bar, 5 µm.

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as transitional ER (tER) [1]. These are relatively immobile domains of 1–2 µm in diameter closely apposed to tubular clusters, also termed pre-Golgi intermediates, vesicular-tubular clusters (VTCs), or ERGIC (ER-Golgi intermediate compartment) [2–5].

Initial transport of cargo out of the ER is mediated by the activity of the small GTPase Sar1 and the cytosolic coat complex known as COPII [6–9]. COPII components interact sequentially: an integral ER membrane protein, Sec12 [10], acts as a specific guanine nucleotide exchange factor (GEF) for the activation of Sar1, a small GTP-binding protein [11–13]; Sar1 is recruited to the ER membrane by Sec12 [10, 14]; the exchange of GDP for GTP on Sar1 determines its activation; the Sec23-Sec24 heterodimer then binds to membrane-anchored Sar1-GTP [11, 15]; coat and vesicle formation is initiated by the binding of a Sec13-Sec31 complex [15, 16], which acts as scaffolding for the newly forming vesicle; GTP hydrolysis on Sar1 dissociates the GTPase from the ER [11], rendering the COPII components labile and easily displaced from a completed vesicle [17].

A second coat complex known as COPI [18–21] whose assembly is regulated by the GTPase Arf1 [22, 23] is required for maturation of pre-Golgi intermediates [24–26]. Arf1 is activated by a GEF sensitive to the fungal metabolite brefeldin A (BFA) [27, 28], a tool often used to block COPI assembly and thus to disrupt trafficking pathways in the early secretory pathway. COPI is also involved in regulating intra-Golgi trafficking, although the nature of this role is unclear [29–35].

The connection between tER and VTCs is not clearly understood. COPII-coated vesicles containing protein cargo may bud on the surface of tER and then fuse with VTCs [3, 8]. Alternatively, tER may mature directly by tubule fusion and fission into VTCs that translocate to the Golgi, and ER-to-Golgi protein transport may then take place without vesicle carriers [32, 36, 37].

Pre-Golgi intermediates are transient discrete entities that move through the action of dynein-driven motors on the microtubule cytoskeleton [38, 39], to fuse with the *cis*-face of the Golgi apparatus and deliver protein cargo through Rab1-dependent tethering and fusion factors [40, 41]. The pre-Golgi compartment may also be the first site of retrieval and recycling of proteins back to the ER [24, 42, 43]. Retrieval of escaped ER proteins as well as retrograde transport of itinerant proteins such as the KDEL receptor is also a function of the *cis*-Golgi [5, 29, 44, 45].

Once arrived at the Golgi, the modality of intra-Golgi transport of secretory cargo is still a matter of debate (reviewed in [46–49]). According to the cisternal maturation model, the Golgi apparatus exists as a polarised organelle where cisternae progress through the Golgi, such that the *cis*-face is newly formed upon fusion of the pre-Golgi compartments and the *trans*-face would represent the oldest portion of the Golgi [1, 49–54]. Alternatively, intra-Golgi trafficking may exploit vesicles as cargo carriers to accomplish movement and sorting of cargo in different compartments of the Golgi apparatus, as postulated in the vesicle carrier model [31, 33, 55–57]. A further model incorporates aspects of both hypotheses such that intra-Golgi transport occurs both upon cisternal maturation and with some movement of vesicle carriers [47, 54, 58]. Another model suggests that cargo moves through permanent or transient continuities (i.e. transport tubules) that connect successive compartments, thereby creating a continuous compartment through which cargo can flow [46, 51, 59, 60].

Transport from the Golgi apparatus to the plasma membrane occurs by post-Golgi carriers [61, 62]. These initiate as tubular extensions from the TGN, which extend out from Golgi membranes and are severed at the farthest end by the action of a dynamin-2 [63]. Post-Golgi transport may require an intact actin cytoskeleton to be efficient, as depolymerisation of actin with cytochalasin B significantly slows export of cargo from the Golgi, while microtubule depolymerisation with nocodazole has no effect on cargo export [61].

Plants

Studies on the dynamics of the plant Golgi are relatively preliminary in comparison with the mammalian counterpart. Plant endomembranes do not label with vital lipophilic dyes that specifically stain the membranes of targeted organelles in mammalian cells [64]. Studies on plant Golgi dynamics using GFP have also suffered a delay with respect to other biological systems due to the presence of a cryptic intron in the wild-type GFP that caused aberrant splicing of the GFP coding sequence such that the mature protein was unable to fluoresce [65, 66]. This phenomenon restricted the use of GFP to RNA viral vectors where expression is not controlled by splicing [67]. It was not until 1997 that Haseloff et al. produced a GFP with altered codon usage, which eliminated the occurrence of aberrant splicing [66]. This opened up the possibility of GFP technology to the study of plant organelle dynamics [68, 69]. The subsequent availability of new GFP spectral variants lacking the cryptic intron has further accelerated the study of plant cell dynamics. Another reason for the comparable delay in the characterisation of plant Golgi dynamics is due to the scarcity of genuine plant Golgi enzymes to be used as markers in vivo. In fact, it was not until GFP was fused to the putative *Arabidopsis* H/KDEL receptor Erd2 (a protein found in mammalian cells to cycle in the ER-to-Golgi pathway while recycling soluble proteins tagged with KDEL back to the ER [44, 70]) and also to the transmembrane domain of a rat sialyl-transferase (a Golgi glycosylation enzyme) [71], and that both chimaeras were found to localise to Golgi stacks by electron microscopy (EM), that the plant

Golgi could be investigated in vivo for the first time [72]. Subsequent identification of genuine plant Golgi enzymes and their fusion to GFP has opened up further the characterisation of the dynamics of the Golgi apparatus in different plant cell systems [73–76].

The organisation of plant Golgi apparatus at the light level appears rather different to the mammalian Golgi (fig. 1B). The plant Golgi apparatus is scattered in the cell as small cisternal stacks in the cortical cytoplasm and within *trans*-vacuolar strands of cytoplasm [72–75]. In leaves, using fluorescent protein constructs, it appears that the individual cisternal stacks of the Golgi apparatus are closely associated with the cortical ER network [72, 77]. These stacks move in an actin-myosin dependent manner over the tubules of the ER [72, 75], in contrast to mammalian cells where microtubules are required for ER-to-Golgi trafficking and Golgi positioning [38, 61]. Movie sequences of the ER-Golgi continuum indicate that movement of the plant Golgi may in part be related to modelling of the underlying ER network through control of ER tubule growth (see movies http://www.brookes.ac.uk/schools/bms/research/molcell/hawes/gfp/gfp.html) [78]. This indicates that the ER and Golgi may behave as one dynamic system, either through direct membrane continuities or through continuous vesicle or tubule formation/fusion reactions [78].

This dynamic organisation of the plant secretory pathway opens up questions about the modality of ER-to-Golgi transport and the distribution of ER export sites. One model originated from observations gathered in BY-2 cells expressing a mannosidase II (a Golgi-resident glycosylation enzyme [79])-GFP chimaera. As the Golgi stacks move, arrest and regain movement after time intervals, it was postulated the discontinuity in Golgi movement is linked to ER-to-Golgi protein transport. This would occur during Golgi arrest, possibly after transient detachment from actin ('stop-and-go model') [75]. Alternatively, Golgi movement in plant cells would allow the Golgi to continually collect vesicles budding from the ER ('vacuum cleaner model') [72].

In yeast and mammalian cells, most of the genes involved in ER-to-Golgi transport have been characterised (see above). The cytosolic coat protein complexes COPI and COPII shuttle proteins between the ER and Golgi. Growing evidence in the plant literature [80–83] indicates that components of the COP systems also operate in protein trafficking in plant cells. Homologues of Sar1, Rab1 and Arf1 have been cloned, and their GTPase-deficient mutants act as *trans* dominant negatives for protein transport [82, 84–86]. Takeuchi et al. established that an Arf1-GFP chimaera localises at organelles, which they consider Golgi [87]. These findings are in accordance with immunolocalisation of COPI components [81, 83] to small vesicles surrounding or budding from cisternae at the *cis* face of the Golgi apparatus in plant cells [81, 83].

The modality of transport of proteins through the plant Golgi is still a matter of debate. Ritzenthaler et al. postulated that intra-Golgi transport in BY-2 cells occurs by cisternal maturation [83]. This was based on results obtained with the fungal metabolite BFA, which collapses Golgi membranes into the ER [88]. A less invasive approach is still needed to establish the nature of the real mechanisms underlying intra-Golgi transport in plants.

Fluorescence recovery after photobleaching

GFP fluorescence has proved invaluable as a visual tool for determining protein location and transport pathways within cells, for investigating the biogenesis and dynamics of organelles, as well as for direct observation of the trafficking of proteins within, and between, living cells. However, a more sophisticated application of GFP for the study of cellular dynamics is based on the ability to irreversibly photobleach the GFP fluorophore and to then measure the recovery of fluorescence by exchange of bleached for unbleached GFP molecules, a process known as fluorescence recovery after photobleaching (FRAP). This technique has been used to assess the structure and connectivity of artificial [89] and biological membranes [90].

A general downside of a FRAP protocol is the development of free radicals, generated by the high-energy laser irradiation, capable of causing damage to the cell. However, GFP is a particularly well-suited fluorescent tag for FRAP experiments. Upon bleaching, the GFP barrel that surrounds the core fluorophore constrains such free radicals from escaping and damaging cellular components, such as proteins and lipids.

The key fundamentals of FRAP are (i) the fluorescence of the fluorochrome (such as GFP) is irreversibly eliminated with an intense photobleaching pulse of laser light; (ii) in the absence of physical constraints, a protein is free to diffuse, i.e. it is able to move randomly by Brownian motion in its environment and (iii) recovery of fluorescence into the bleached area is the result of exchange due to diffusion of unbleached molecules with bleached ones in the same area [90].

There are two types of FRAP protocols, quantitative and qualitative. Qualitative FRAP allows protein movement to be followed in vivo, within a defined area. This will produce high-quality images that show a global fluorescence recovery within the bleached area but no data useful for quantifying protein mobility. The quantitative protocol, on the other hand, allows estimation of a diffusion coefficient, calculation of the mobile fraction and the time of recovery of fluorescence after bleaching of a region of interest (ROI). The mobile fraction is the percentage of fluorescent protein that diffuses into a bleached

area, and a diffusion coefficient is a measure of movement of the mobile pool of fluorescent proteins over time. In FRAP experiments, the half-time of recovery indicates the time required for the fluorescence to reach 50% of a plateau of intensity.

With the advent of two-colour GFP imaging [91], another application of FRAP is to use photobleaching to visualise protein trafficking or flux through a two-colour bleaching protocol [78, 92, 93]. Proteins or signals to be visualised should be tagged with either the cyan- (CFP) or yellow- (YFP) fluorescent protein and coexpressed in the same cells [91]. The two fluorochromes may be targeted to the same organelle or even two chimaeras of the same protein. In this FRAP protocol, YFP and CFP molecules have to be excited together, using a multi-tracking mode of a confocal microscope with a 514-nm laser line to excite YFP and a 458-nm (or 413-nm) laser line to excite CFP. YFP is selectively photobleached using the 514-nm laser line at maximum power, leaving CFP fluorescence unaltered. This technique is a great advance of the FRAP technology as it exploits CFP as a visual reference to follow the dynamics of a targeted organelle, and simultaneously to follow and quantify YFP fluorescence recovery. In addition, when YFP and CFP chimaeras of the same protein are coexpressed, fluorescence recovery may be tracked by image differencing by subtracting the image of the bleached fluorochrome from that of the unbleached fluorochrome, a technique called fluorescence localisation after photobleaching (FLAP) [93].

Another application of photobleaching is to reduce fluorescence from background noise to reveal faint populations of fluorescent proteins [34]. Alternatively, photobleaching of a large pool of protein, for example on the plasma membrane, allows visualisation of organelle dynamics inside the cell usually masked by the plasma membrane pool. For example, a protein that cycles between the Golgi and plasma membrane might then be visualised in translocating intermediates [94].

Finally, fluorescence loss in photobleaching (FLIP) investigates fluorophore mobility and continuity between fluorescent organelles [95]. FLIP is similar to FRAP in that a region of interest is photobleached with a high-power laser; however, unlike FRAP, this is repeatedly bleached over time to deplete the entire fluorescent pool. With this technique it is possible to assess the spatial continuity between two organelles if photobleaching of one depletes the entire fluorescence of the other or even to determine the free diffusion of a protein within a subcellular compartment.

FRAP applied to mammalian Golgi dynamics

FRAP as a tool to answer biological questions has found applications in both animal and plant cells. However, this technology has uncovered noticeable differences between the dynamics of the Golgi in the two systems.

In mammalian cells, FRAP has been applied to investigate diffusion, exchange, kinetics of proteins passing through the Golgi apparatus.

Diffusion of proteins in the Golgi

Initial visualisation of the mammalian Golgi in vivo was based on the application of fluorescent lipid derivatives, such as NBD ceramide, to label the membrane as there was no way to label proteins while maintaining cell viability [96]. The usefulness of the vital stain NBD ceramide was restricted by its metabolism into sphingolipids that were then trafficked to the plasma membrane, making it only a transient marker for the Golgi [97].

However, it allowed visualisation of tubulovesicular processes emerging from the *trans*-Golgi network (TGN), which either formed transport intermediates or could fuse with adjacent *trans*-Golgi elements in a microtubule-dependent manner, forming a reticular network [98]. By using FRAP, Cooper et al. were able to show that these tubulovesicular processes formed pathways allowing diffusion of membrane lipids between joined *trans*-Golgi elements, although not between obviously separate, discrete elements [98]. This study therefore demonstrated for the first time that the dynamic processes at the TGN are not only required for post-Golgi transport but also for connectivity within the Golgi itself. Storrie et al. used indirect labelling to visualise the movement of a cargo protein through the secretory pathway in vivo [99]. Cells infected with the ts045 mutant of vesicular stomatitis virus (VSV-ts045) were held at 40 °C to accumulate the virus glycoprotein (ts045-G or VSVG) in the ER. Microinjection of rhodamine-conjugated Fab fragments of a monoclonal antibody against the ts-045-G cytoplasmic tail enabled tracking of the protein through the cell. FRAP of a thin strip across the Golgi in cells labelled with rhodamine label revealed that VSVG was able to diffuse in Golgi and *trans*-Golgi membranes at rates faster than those found for proteins on the plasma membrane, i.e. it is not constrained by protein complexes [99, 100].

A real boost to FRAP application was given by the advent of GFP technology. In 1996, Cole et al. published a seminal paper not only using GFP as a marker for membrane trafficking in mammalian cells for the first time, but also demonstrating the biophysical potential of GFP for use in live cell imaging and photobleaching techniques [95]. This enabled analysis of the behaviour of cellular proteins not possible by previous, more invasive, means such as biochemistry and EM. The authors created a number of chimaeric Golgi membrane proteins, including Erd2 and mannosidase II (Man2 [101]), by fusing the protein cod-

ing sequence to GFP-encoding DNA and then expressing the constructs in mammalian cell lines. These chimaeric proteins were found to correctly localise to the Golgi. Furthermore, a chimaera comprising just the transmembrane domain of galactosyltransferase fused to GFP was also found to localise to the Golgi, showing that targeting signals present in that domain alone are responsible for Golgi localisation [95, 102, 103]. In fact it appears that the length of the transmembrane domain of a protein, and not its amino acid content, is sufficient to determine Golgi localisation [102, 103].

At this time, the vesicular trafficking model of intra-Golgi transport predicted that Golgi-localised enzymes were found in the cisternae of the stack, while transiting cargo was ferried between cisternae by vesicles [31, 104]. A dilemma was how the enzymes were prevented from entering the vesicles. One hypothesis was that 'kin recognition' between fellow enzymes would cause them to interact and cluster together, creating large heterooligomers unable to fit into the small translocating vesicles [105]. These protein complexes might even be further tethered to an underlying Golgi matrix holding the cisternae together in a stacked formation [105]. This proposal was based on the observation that overexpression of a Golgi enzyme misdirected to the ER caused relocalisation of other enzymes to the ER [101]. A FRAP approach on Golgi proteins tagged with GFP elegantly provided the first evidence for disproving the kin-recognition model [95]. When Cole et al. analysed the dynamics of the GFP-tagged Golgi membrane proteins in living cells, they bleached small strips of fluorescence across the Golgi stack and looked for recovery [95]. The kin recognition model predicted that the membrane proteins would be held in a stable conformation. However, the GFP chimaeras were found to rapidly recover the fluorescence to the bleached region of interest with very high mobile fraction (85–98%) and diffusion coefficient. This indicated that the GFP chimaeras were freely diffusible in the lipid bilayer within the Golgi cisternae and that they were not aggregating or being slowed by interactions with other proteins. Chimaeric GFP proteins of the p24 family also exhibit fast diffusion through Golgi membranes following photobleaching of a small region [106]. Aluminium fluoride (AlF) is an activator of heterotrimeric G proteins [107] and causes irreversible binding of peripheral protein complexes to the Golgi membrane [23, 108, 109]. In FRAP experiments on cells pretreated with AlF for 10 min, virtually complete immobilization of all of the GFP chimaeras in Golgi membranes was observed [95, 106]. This showed that only when peripheral proteins are unable to release from membranes are Golgi proteins immobilized.

To investigate the extent to which Golgi membranes are continuous, the same study [95] used a FLIP protocol for first time. By repeatedly photobleaching a strip across the Golgi, the authors could entirely deplete Golgi fluorescence extending outside the strip. Different regions of the Golgi lost fluorescence at different rates, possibly due to differences in connectivity, but overall extensive lateral diffusion occurred between Golgi stacks. Thus, the chimaeras could diffuse rapidly and freely in Golgi membranes, suggesting that Golgi targeting and retention of these molecules does not depend on protein immobilization.

Measuring diffusion of Golgi components was also used to determine the fate of the Golgi membranes during mitosis [110]. The tightly packed pericentriolar localisation of the Golgi of interphase cells becomes dispersed during mitosis and then recoalesces as cells enter interphase once more. The nature of these dispersed membranes has been much disputed [111, 112]. One model argues that the Golgi becomes absorbed into the ER, taking advantage of preexisting cycling pathways [110, 111], while the other proposes that the Golgi is dispersed into vesicles distributed throughout the cell [112]. Zaal et al. used FRAP, in the first instance, of a Golgi membrane protein galactosyl transferase (GalT) tagged with GFP to investigate its mobility in mitotic cells [110]. They found that the rate of diffusion of the membrane protein in the dispersed mitotic membranes was equivalent to that found for the same chimaera when expressed in the ER in interphase cells, i.e. in a continuous membrane system. Since this does not exclude the possibility that vesicles would be able to diffuse very fast through the cytosol of the mitotic cell, the authors then compared this mobility to that of Golgi lipids in mitotic cells. If the Golgi redistributes into vesicles in a mitotic cell, then the fluorescence of Golgi-resident proteins and lipids would be expected to recover at identical rates into a bleached region since they would be on the same carrier, rather than moving as separate components of a continuous membrane system. However, a fluorescent lipid analogue BODIPY-ceramide was found by FRAP to have a diffusional mobility much greater (10-fold faster) than the GFP-tagged Golgi protein, consistent with the different mobility expected between a protein and a lipid in an interconnected membrane system [110]. BODIPY-ceramide dynamics in a mitotic cell also matched those found for the dye when redistributed to the ER, upon treatment with BFA, in interphase cells. These data are therefore not consistent with a vesicle model for the Golgi in mitosis. Furthermore, when GalT-GFP was repetitively photobleached using the FLIP protocol in mitotic cells, the diffuse label was completely depleted, demonstrating that it is capable of diffusing throughout the membranes in metaphase cells, and therefore indicative of localisation to the ER [110]. In mitotic sea urchin embryo cells, FLIP of Golgi markers including GalT-GFP and a KDEL-receptor-GFP (Erd2) also revealed their localisation to a continuous membrane system [113]. This suggests that relocalisation of the

Golgi membranes to the ER may represent a common mechanism for the division of the Golgi between daughter cells in eukaryotes.

Exchange

FRAP has been pivotal in defining the mammalian Golgi as a dynamic organelle in the secretory pathway through analysis of the behaviour of the Golgi's constituent parts. There are many different types of proteins associated with the Golgi that are required to accomplish its many functions. Membrane-bound proteins include the glycosylation enzymes, required for the maturation of secretory cargo, as well as putative cargo receptors such as ERGIC-53, the p24 family and Erd2 (KDEL receptor) that cycle between the ER and the Golgi. Then there are the peripherally associated proteins, such as coat proteins (COPI and the clathrin/adaptor complex), the scaffold proteins thought to hold the Golgi in its characteristic stacked formation (e.g., GM130, GRASP65 [114, 115]) and signalling molecules such as PLD1, PKA, MEKK2 and PI-3K [116–120].

While Golgi membrane proteins had been found to be freely diffusible within the Golgi apparatus [95] and itinerant proteins such as ERGIC-53 and Erd2 had been found to cycle constitutively between the ER and the Golgi [5, 44], the Golgi glycosylation enzymes were still considered held within the boundaries of the Golgi as a whole. It had been observed that upon treatment of cells with nocodazole (which causes microtubules to depolymerise), Golgi membrane proteins became localised to small punctate structures [121, 122]. It was proposed that this was caused by fragmentation and dispersal of the Golgi [122, 123]. These nocodazole-induced structures were found to resemble mini-Golgi bodies by EM [124]. When cells expressing GFP chimaeras of Golgi glycosylation enzymes were treated with nocodazole, FRAP of these mini-stacks showed that these membrane proteins were not constrained to the individual structures since the fluorescence recovered, suggesting that resident proteins were able to exchange between stacks, potentially via the ER, although exchange by trafficking of small vesicles from other mini-stacks could not be excluded [125]. However, when the whole Golgi was photobleached immediately after addition of nocodazole, fluorescence was still found to accumulate into mini-stacks in the absence of new protein synthesis [110].

In these experiments, recovery of fluorescence indicated the existence of another cellular pool of the Golgi enzymes, which was thought to be the ER. Moreover, FRAP experiments indicated that an exchange of the photobleached molecules with an unbleached pool enabled the fluorescence recovery by cycling pathways between the ER and the Golgi. That Golgi enzymes cycle through the ER was confirmed by microinjection of a dominant GTP-bound Sar1 mutant, which blocks protein ER exit. Blockage of exit from the ER causes Golgi enzymes to gradually accumulate in the ER as they recycle from the Golgi [110, 125]. To show that Golgi enzymes still cycle constitutively in the absence of cellular perturbants, the Golgi pool of GalT-GFP was selectively photobleached in untreated cells (fig. 2A). Fluorescence was found to recover to the area within 20 min, indicating exchange with the ER pool. Conversely, if the total cellular fluorescence outside of the Golgi (i.e. the ER pool) was photobleached, it was found to recover with comparable decrease in total Golgi fluorescence [110]. The purpose of this cycling pathway in interphase cells is not clear but may underlie maintenance of Golgi structure [32, 110].

In contrast to the Golgi enzymes, other membrane proteins associated with the Golgi, such as KDEL receptor, are required to cycle between the ER and the Golgi in order to fulfil their proposed functions, i.e. to retrieve escaped soluble KDEL-labelled ER proteins back from the Golgi [44]. Other proteins such as ERGIC-53 and the p24 family of proteins are also thought to be cargo receptors and cycle between the ER and the Golgi [5, 126]. Unsurprisingly, therefore, FRAP of the Golgi fluorescence in cells expressing GFP chimaeras of these proteins shows that recovery is much faster (within 4 min) than that of the glycosylation enzymes, indicating faster cycling times within the early secretory pathway [26, 127].

FRAP of membrane-associated proteins has also led to the identification of novel trafficking pathways [94]. GFP targeted to membranes through a glycosyl phosphatidylinositol (GPI) anchor, or another endogenous GPI-linked protein tagged with GFP, CD59-GFP, are both found predominantly on the plasma membrane, but there is also a significant Golgi pool. FRAP of the Golgi pool in the absence of protein synthesis showed that the photobleached GFP chimaeras were exchanging with the plasma membrane pool, enabling recovery of fluorescence to the Golgi. Nichols et al. then showed that this recovery was independent of clathrin-mediated endocytosis, thereby indicating that there is a route from plasma membrane to the Golgi that bypasses the endocytic pathway [94].

The behaviour of the peripheral Golgi coat proteins is very different from the membrane-associated Golgi proteins. COPI is a cytosolic protein complex that is recruited onto the membrane by the small GTPase Arf1, where it then facilitates membrane budding and fission of transport intermediates. FRAP of Golgi membranes labelled with either Arf1-GFP or εCOP-GFP (a component of COPI) shows that there is rapid exchange between cytosolic and membrane-bound pools of the proteins [34, 128]. Cycling is dependent on the guanine nucleotide binding cycle of Arf1: when the cells additionally express a dominant positive mutant of Arf1, i.e. it is held in the GTP-bound state, both Arf1 and COPI become irre-

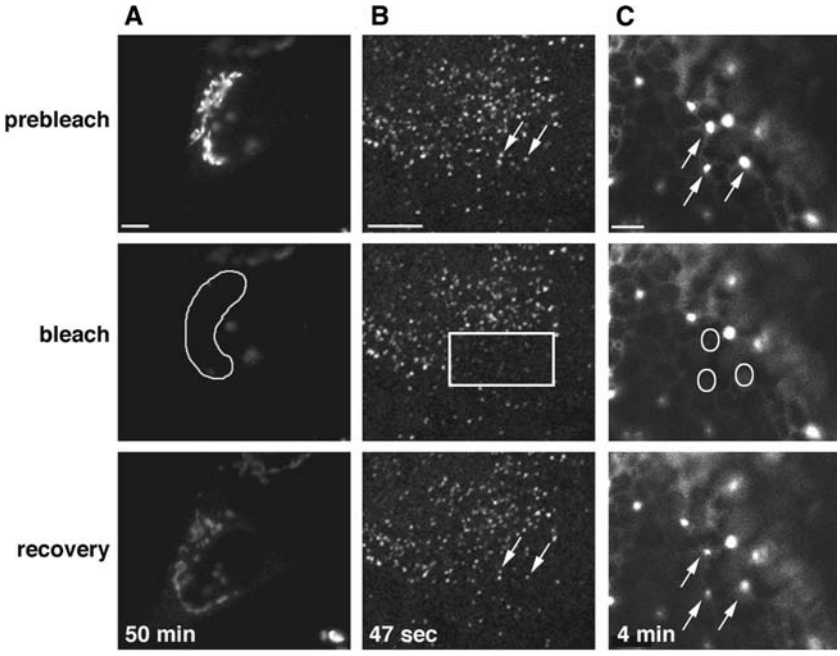

Figure 2. Demonstration of FRAP in living cells. (*A*) NRK cells stably expressing GalT-YFP to label the mammalian Golgi. The Golgi region was photobleached with high-intensity laser, and recovery was monitored over time at low laser intensity. (*B*) NRK cells stably expressing Sec13-YFP to label mammalian ER exit sites. A region of interest encompassing a number of ER exit sites was photobleached, and recovery was monitored over time. (*C*) Photobleaching of Erd2-GFP fluorescence localised in the Golgi (arrows) of tobacco leaf epidermal cells. The cells were treated as described in [78], with actin and microtubule depolymerising agents Fluorescence recovery indicates that movement along the actin/microtubule cytoskeleton is not required for ER-to-Golgi membrane protein transport. Scale bar, 5 µm. (*A*) and (*B*) reproduced from The Journal of Cell Biology (2001) 155: 557–570 by copyright permission of The Rockefeller University Press.

versibly bound to Golgi membranes and no recovery is seen when the fluorescence is photobleached [34, 128].

The scaffold, or matrix, proteins (including GM130 and GRASP65) are peripherally associated Golgi proteins that are thought to hold the Golgi in its stacked conformation [114, 115]. Expression of a full-length GRASP65-GFP chimaera labelled the Golgi [129]. When this was selectively photobleached, recovery to the membranes was very rapid with significant fluorescence visible within 1 min, similar to the recovery seen for the coat proteins [26]. To determine whether the fast recovery after photobleaching was due to its rapid cycling through ER-Golgi membrane pathways or to its binding and releasing from Golgi membranes, Ward et al. examined the effect of microtubule disruption on the recovery kinetics [26]. As microtubules serve as tracks for the ER-to-Golgi transport of membrane-bound intermediates [38], microtubule disruption would only be expected to inhibit GRASP65-GFP dynamics if the protein were rapidly cycling to and from the Golgi via membrane trafficking pathways, but would have no effect if GRASP65-GFP were binding and releasing from Golgi membranes. When the Golgi fluorescence was photobleached immediately after depolymerisation of microtubules (i.e. before Golgi fragmentation), recovery of GRASP65-GFP was unaffected. Under the same conditions, similar results were found for εCOP-GFP, which does not require membrane cycling pathways to associate with the Golgi [26, 34], but recovery of a membrane-bound protein, p58-GFP, was significantly impaired. This therefore showed that the scaffold proteins do not form a stable matrix but instead associate dynamically with Golgi membranes in a manner reminiscent of the coat proteins.

The cargo protein VSVG tagged with GFP has proved a very important marker for membrane traffic. It has enabled characterisation for the first time not only of ER-to-Golgi transport intermediates [38] but also post-Golgi intermediates [61]. In both cases, rather than being small vesicular carriers, VSVG-GFP showed that the cell uses large pleiomorphic structures to translocate material through the cytosol. In order to show that the VSVG-GFP-containing tubular structures budding off the ER are truly destined for the Golgi, fluorescence in the Golgi was selectively photobleached [38]. Trafficking intermediates were then visualised translocating into the Golgi region, and concomitant recovery of fluorescence to the Golgi was found [38].

White et al. have used the development of two-colour GFP imaging [91] to label the Golgi with T2-CFP (the stalk region of *N*-acetylgalactosaminyltransferase-2 tagged with cyan fluorescent protein [125]), while visualising translocating cargo dynamics with VSVG tagged

with yellow fluorescent protein [92]. FRAP of VSVG-YFP, while simultaneously imaging unbleached T2-CFP to follow the morphology of Golgi membranes, showed that at the resolution offered by a confocal microscope, Golgi membranes do not appear to change while VSVG traffics through, and exits from, the organelle [92].

The debate over the mechanism of Golgi replication in mitotic mammalian cells as discussed above has led to two models to explain the reformation of the Golgi in the daughter cells. Merging of the Golgi with the ER during mitosis would enable a new Golgi to grow out of the ER by de novo synthesis [26, 110], whereas the alternative model suggests that preexisting Golgi in the form of the mitotic vesicles and fragmented membranes would fuse back together, i.e. that the Golgi is an autonomously replicating organelle [130–134]. Many approaches have been used to address this question. These have centered mainly on finding whether non-mitotic cells are able to regrow a Golgi. The fungal metabolite BFA causes reversible absorption of Golgi membranes by the ER [88]. However, some components do not fuse with the ER; for example the matrix proteins and cycling membrane proteins, such as ERGIC-53, label small punctate structures scattered through the cell [114, 135, 136]. Similarly, when mammalian cells express the activated Sar1 mutant Sar1[H79G], the glycosylation enzymes are retained in the ER, while matrix and cycling proteins such as ERGIC-53 and KDEL receptor are found in separate structures [26]. The nature of these structures has been the subject of much discussion [26, 131]. They have been termed 'Golgi remnants' that are left behind as residual Golgi membranes that would then seed the growth of new Golgi upon release of the transport block [131, 137]. However, Ward et al. showed that upon treatment with BFA, these structures were not originating from the Golgi but appear in the cytosol without tracking out from the Golgi and therefore must form de novo [26]. They colocalise with ER exit sites as defined by COPII label [26]. Subunits of the COPII coat complex required for ER exit exhibit fast membrane-cytosol exchange as visualised by FRAP of ER exit sites expressing GFP chimaeras (fig. 2B) [25, 26], in a manner similar to COPI [26, 34, 128]. Upon treatment with BFA, FRAP experiments of COPII components proved that these are still cycling at ER exit sites [26]. The subsequent finding, by FRAP, that ERGIC-53 chimaeras, as well as matrix proteins, are still cycling either between the structures and the ER, or exchanging with a cytosolic pool, confirmed that the BFA-induced 'Golgi remnants' are at ER exit sites, and are able to recruit only certain classes of proteins [26]. Release of the COPI block (after removal of BFA by washout) allows these membranes, which closely resemble VTCs [26, 138], to mature into pre-Golgi intermediates and reform the Golgi in the perinuclear region. Expression of the dominant negative Sar1[T39N] (GDP-bound Sar1 mutant) [26] or very high levels of Sar1[H79G] (GTP-bound Sar1 mutant) [139] prevent formation of COPII-labelled ER exit sites, and all Golgi components become redistributed to the ER.

Thus, FRAP analysis had led to unique understanding of the dynamics of recycling and resident Golgi membrane proteins, coatomers and matrix proteins. It appears that all these components of the Golgi are dynamically associated with this organelle. These data and the observation that when the protein cycling pathways are disrupted, Golgi integrity breaks down, and Golgi components are redistributed to the site of the transport block, suggest that when conditions for a Golgi reformation apply, the Golgi may then reform de novo.

FRAP has also been used to investigate processes underlying cellular differentiation. During skeletal muscle cell differentiation from myoblasts to myotubes, the Golgi undergoes a dramatic reorganization, such that instead of a compact perinuclear compartment, it is dispersed into elements that form a fenestrated belt around the myotube nuclei. FRAP analysis of myoblasts expressing mannosidase II labeled with GFP after Golgi reorganization demonstrated that Golgi-localised proteins are still cycling between the Golgi elements and the ER [140]. The mechanism for Golgi dispersal may follow a Golgi-ER cycling route similar to that found in undifferentiated cells on treatment with nocodazole [140].

Kinetics

Quantitative time-lapse imaging data of single cells expressing GFP-tagged molecules have been used to address the kinetic properties of secretory transport, including how long a protein resides in a particular compartment and the rate of transfer of a protein between compartments. This approach was first used to investigate the behaviour of VSVG-GFP as it translocates the secretory pathway. Hirschberg et al. were able to determine the rates of VSVG-GFP influx into, and efflux from, different secretory compartments [61]. They found that even at very high concentration of cargo (e.g. when VSVG is first released from the 40 °C temperature block and begins to exit the ER), efflux from the ER occurs at a consistent rate, indicating that the machinery is not saturated. The kinetic analysis also indicated that there would be no lag as VSVG translocated the Golgi, contrary to the predictions of the cisternal maturation model [141]. These quantitation experiments required data sets obtained simply by visualization of VSVG-GFP as it is transported through the cell. However, FRAP of the Golgi pool of GFP chimaeric proteins has enabled quantitation of their compartmental exchange. For example, kinetic modeling of FRAP experiments revealed that an average GalT-GFP molecule cycles between Golgi and ER every 85 min, residing in the Golgi for approximately 58 min and in the

ER for 27 min [110]. In contrast, an itinerant Golgi protein related to ERGIC-53, VIP36-GFP, was found to have a half-life for residency in the ER of 113 min but only 1.67 min in the Golgi [127], accounting for the chimaera's steady-state distribution of 13% Golgi/87% ER. In the Golgi-to-plasma membrane cycling pathway discovered for the GPI-anchored proteins, it was found that on average GPI-GFP molecules reside in the plasma membrane for ~200 min and in the Golgi complex for ~9 min [94].

Kinetic analysis of the lifetime of residency of the coat proteins εCOP-GFP and Arf1-GFP on Golgi membranes, as measured by both FRAP and release upon addition of BFA, has given important information about the enzyme's kinetics and the behaviour of the COPI coat and its interactions with the Golgi membrane [34]. While activated Arf1 is required to bring COPI to the membrane, COPI remains bound once associated with the membrane even after Arf1 has hydrolysed its GTP and dissociated. This uncoupling of COPI from Arf1, and the ability for these proteins to exchange at 4 °C, below the temperature for vesicle budding, suggests more than the simple formation of a vesicle coat, and it seems possible that Arf1 and COPI are together required for generating membrane domains that might then become transport intermediates [34].

Plant Golgi dynamics analysed by FRAP

FRAP, as with many other sophisticated applications of GFP and its fluorescent derivatives, is a relatively novel technique for the study of the endomembrane system in plant cells. Brandizzi et al. reported for the first time in 2002 on the movement of membrane proteins in vivo from the ER to the Golgi in tobacco leaves [78]. In this study, EM showed that Golgi apparatus has an apparent continuity with the ER. Such a vicinity raised questions on the nature of protein movement from the ER to the Golgi. It is possible, on the basis of the EM observation, that protein transport to the Golgi could occur by diffusion. To solve this issue, FRAP experiments were performed on leaf tissues expressing GFP fused to either Erd2 (H/KDEL receptor) or the membrane domain of a rat sialyl transferase (fig. 2C). These experiments showed that recovery of fluorescence to the bleached Golgi is energy dependent, and therefore ER-to-Golgi transport in plant cells could not be a process of passive diffusion [78].

In tobacco plant cells, Golgi are distributed on the ER and alternate motile and arrested phases [72, 77, 85]. The movement of Golgi bodies over the ER is driven by actin-myosin motors [72, 77, 78]. To investigate whether an actin or microtubule cytoskeleton participates in protein transport towards the Golgi, leaf tissues were treated with cytoskeleton depolymerising agents. In the absence of an intact actin cytoskeleton, Golgi bodies stopped moving, and when a FRAP experiment was carried out in these conditions, Golgi stacks recovered their fluorescence within 5 min of the bleaching event [78]. This shows that actin is clearly not required for ER-to-Golgi trafficking even though it is required for Golgi movement.

To investigate the involvement of microtubules in the movement of membranes towards the Golgi, leaf tissues were treated with colchicine to depolymerise microtubules. In the absence of microtubules, Golgi bodies were still mobile. Therefore, the concomitant use of an actin depolymerising agent was still required in order to perform FRAP on immobilised Golgi. Again, Golgi bodies regained fluorescence within 5 min of the bleaching events [78].

FRAP experiments performed in the absence of intact microtubules and/or actin on membrane proteins like Erd2-GFP and a transmembrane domain of a rat sialyl-transferase fused to GFP (ST-GFP) [72], which locate all over the Golgi and towards the *trans* areas of the Golgi, respectively [72], did not highlight substantial differences in the time of recovery upon photobleaching, suggesting that Golgi components that behave differently in mammalian cells are able to exchange between the plant ER and Golgi with similar kinetics. The recovery time of these two proteins also did not differ from the recovery time of a glycosylated form of ST-GFP [78, 85].

These results highlight differences between the plant and mammalian Golgi. In mammalian cells, microtubules are essential for translocation of pre-Golgi intermediates to the Golgi apparatus, but in the absence of microtubules (by treatment with nocodazole) the emergence of mini-Golgi stacks at ER exit sites reestablishes secretory flow from the ER to the plasma membrane [32, 124]. In plants, the cytoskeleton does not seem to be essential for protein transport to the Golgi. However, there are still two open questions. It is still unknown in plant cells whether protein transport to the Golgi would be faster in the presence of an intact cytoskeleton and whether the protein transport to the Golgi occurs during movement or only when Golgi are immobile on the ER. It is possible in fact that upon use of actin-depolymerising agents, Golgi stacks halt on ER export sites. At present we cannot rule out that protein transport to the Golgi may also occur during the motion phases of the stop-and-go pattern as well as the arrest phase over the ER.

The results from Brandizzi et al. are in accordance with those of Saint-Jore et al. [77, 78]. In the latter case BFA was used to investigate the involvement of the cytoskeleton in transport to and from the Golgi with some of the Golgi markers used by Brandizzi et al. [78]. In mammalian cells BFA induces redistribution of the Golgi membrane proteins to the ER [88]. After treatment with BFA, fluorescent Golgi markers expressed in tobacco

epidermal leaves and BY-2 cells are redistributed in the ER [72, 77, 83]. This effect is reversible upon BFA washout [77, 142, 143]. Saint-Jore et al. proved that in the absence of an intact actin and microtubule cytoskeleton, the BFA effect and BFA washout effect were still taking place [77], indicating that movement of proteins to and from the Golgi are cytoskeleton independent.

The effect of BFA on ER/Golgi trafficking in plant cells in vivo was further analysed by FRAP of Golgi double-labelled with YFP and CFP chimaeras of the same protein [78]. YFP was selectively bleached and the integrity of the Golgi stacks, within the limited resolution offered by a confocal microscope, was simultaneously monitored with CFP fluorescence. When either AtErd2-YFP or ST-YFP was bleached in the presence of BFA, but before redistribution of fluorescence into the ER, no recovery of fluorescence was observed in 50% of ST-YFP and 40% of Erd2-YFP labelled stacks. During the recovery period, the CFP signal indicated that photobleaching does not appear to damage the Golgi. Moreover, the double expression results indicate that BFA can inhibit forward transport of proteins to the Golgi before any morphological change in the Golgi, detectable within the resolution of the light microscope, and Golgi membrane reabsorption into the ER, can occur. An explanation for these results may reside in the rapid loss of the COPI coat from Golgi membranes due to BFA inhibition of the Arf1 guanine nucleotide exchange factor [27, 28, 83, 144]. In mammalian cells, BFA treatment has been shown to prevent Golgi enzymes and some cargo from being recruited to ER exit sites, even though the Sar1-COPII system is still operational at these sites [26, 135]. This finding has led to the proposal that the sequential activity of the Sar1-COPII and Arf1-coatomer systems jointly serve to form and maintain Golgi structures [24, 25], whose components continuously circulate through the ER [26]. In this view, Arf1-coatomer is required for forward trafficking out of the ER due to its role in differentiating ER exit domains formed by the Sar1-COPII system. Without the joint activities of both Sar1-COPII and Arf1-coatomer, forward trafficking into the Golgi cannot occur. BFA block on forward transport of AtErd2-GFP and ST-GFP to the Golgi provides important support for this model.

FRAP: where to in the future?

FRAP technology is a key tool to answer questions about the dynamics of cellular organelles. FRAP opens up new and exciting perspectives for investigating dynamics of vital organelles and addresses questions about protein mobility, membrane connectivity and movement in vivo.

GFP-based FRAP is less than a decade old, but it has revolutionised the study of the Golgi in vivo and the proteins that dictate Golgi morphology and physiology. It was developed to bridge the gap between visualisation of a dynamic process and its quantification, and without this technology many processes could not have been otherwise verified and investigated. In this respect, FRAP has proved an invaluable tool to answer fundamental biological questions regarding the dynamics of mammalian and plant Golgi. The application of FRAP to Golgi proteins in both animal and plant cells has been used to demonstrate not only that Golgi proteins are not static/restrained within the organelle, but also the connectivity between organelles within the secretory pathway, i.e. the extent to which the Golgi is in flux with both the ER and plasma membrane. In mammalian cells, FRAP has also been used to elucidate the distribution of Golgi components in both mitosis and interphase and in the presence of cellular perturbants. It has enabled identification of new pathways and has revealed that the Golgi has no stable scaffold. In addition, it has added insight into the mechanisms of coat protein function.

FRAP technology is in continuous development. With the production of GFP spectral variants such as CFP and YFP, FRAP has been adapted to answer new biological questions. In the future, it is likely that two-colour FRAP will be applied to cells more frequently. In particular, this will be particularly useful to carry out FRAP experiments on motile organelles. For example, FRAP of yeast or plant Golgi may now be performed in a more natural environment. It will no longer be necessary to stop Golgi movement with cellular perturbants to perform a single-colour FRAP, as double labelling of Golgi with YFP and CFP will enable moving Golgi to be followed by imaging CFP fluorescence, while quantifying YFP fluorescence after photobleaching at the same time. Another application will be to label a resident protein, for example a Golgi enzyme, and then analyse the behaviour of cargo as it traverses the Golgi to identify exit and entry domains [60, 92].

Finally, the recent development of a photoactivatable GFP [145] will provide a complementary approach to dissecting organelle dynamics. This spectral variant of GFP (PA-GFP) is activated by high-level 413-nm laser irradiation, rather than photobleached, such that it can then be visualised with 488-nm excitation. This enables activation of a specific pool of GFP-tagged proteins, which can then be tracked through the cell, in effect a real-time pulse-chase mechanism. Use of PA-GFP avoids the problem of a potential pool of unfolded GFP that might create later fluorescence [110, 132], as only the activated molecules will fluoresce. In addition, once activated, the pool of fluorescent protein is then stable for long periods of time. This allows monitoring of longer-term cellular changes not possible through standard FRAP applications, which require protein translation inhibitors to prevent production of new GFP-tagged molecules.

FRAP is a state-of-the-art technology in continuous development, and microscopes and software are becoming exponentially more powerful. It may not be long before we will witness the development of more sophisticated imaging facilities able to provide a strong technological platform to apply different combinations of FRAP conditions and to answer simultaneously multiple biological questions inside just a single cell.

1 Bannykh S. I. and Balch W. E. (1997) Membrane dynamics at the endoplasmic reticulum-Golgi interface. J. Cell Biol. **138:** 1–4
2 Saraste J. and Kuismanen E. (1992) Pathways of protein sorting and membrane traffic between the rough endoplasmic reticulum and the Golgi complex. Semin. Cell Biol. **3:** 343–355
3 Bannykh S. I., Rowe T. and Balch W. E. (1996) The organization of endoplasmic reticulum export complexes. J. Cell Biol. **135:** 19–35
4 Hammond A. T. and Glick B. S. (2000) Dynamics of transitional endoplasmic reticulum sites in vertebrate cells. Mol. Biol. Cell **11:** 3013–3030
5 Hauri H.-P., Kappeler F., Andersson H. and Appenzeller C. (2000) ERGIC-53 and traffic in the secretory pathway. J. Cell Sci. **113:** 587–596
6 Novick P., Ferro S. and Schekman R. (1981) Order of events in the yeast secretory pathway. Cell **25:** 461–469
7 Kaiser C. A. and Schekman R. (1990) Distinct sets of *SEC* genes govern transport vesicle formation and fusion early in the secretory pathway. Cell **61:** 723–733
8 Schekman R. and Orci L. (1996) Coat proteins and vesicle budding. Science **271:** 1526–1533
9 Kuehn M. J. and Schekman R. (1997) COPII and secretory cargo capture into transport vesicles. Curr. Opin. Cell Biol. **9:** 477–483
10 Barlowe C. and Schekman R. (1993) *SEC12* encodes a guanine-nucleotide-exchange factor essential for transport vesicle budding from the ER. Nature **365:** 347–349
11 Yoshihisa T., Barlowe C. and Schekman R. (1993) Requirement for a GTPase-activating protein in vesicle budding from the endoplasmic reticulum. Science **259:** 1466–1468
12 Barlowe C., d'Enfert C. and Schekman R. (1993) Purification and characterization of SAR1p, a small GTP-binding protein required for transport vesicle formation from the endoplasmic reticulum. J. Biol. Chem. **268:** 873–879
13 Kuge O., Dascher C., Orci L., Rowe T., Amherdt M., Plutner H. et al. (1994) Sar1 promotes vesicle budding from the endoplasmic reticulum but not Golgi compartments. J. Cell Biol. **125:** 51–65
14 Weissman J. T., Plutner H. and Balch W. E. (2001) The mammalian guanine nucleotide exchange factor mSec12 is essential for activation of the Sar1 GTPase directing endoplasmic reticulum export. Traffic **2:** 465–475
15 Salama N. R., Yeung T. and Schekman R. W. (1993) The Sec13p complex and reconstitution of vesicle budding from the ER with purified cytosolic proteins. EMBO J. **12:** 4073–4082
16 Salama N. R., Chuang J. S. and Schekman R. W. (1997) *SEC31* encodes an essential component of the COPII coat required for transport vesicle budding from the endoplasmic reticulum. Mol. Biol. Cell **8:** 205–217
17 Barlowe C., Orci L., Yeung T., Hosobuchi M., Hamamoto S., Salama N. et al. (1994) COPII: a membrane coat formed by Sec proteins that drive vesicle budding from the endoplasmic reticulum. Cell **77:** 895–907
18 Duden R., Griffiths G., Frank R., Argos P. and Kreis T. E. (1991) β-COP, a 110 kd protein associated with non-clathrin-coated vesicles and the Golgi complex, shows homology to β-adaptin. Cell **64:** 649–665
19 Serafini T., Stenbeck G., Brecht A., Lottspeich F., Orci L., Rothman J. E. et al. (1991) A coat subunit of Golgi-derived non-clathrin-coated vesicles with homology to the clathrin-coated vesicle coat protein β-adaptin. Nature **349:** 215–220
20 Waters M. G., Serafini T. and Rothman J. E. (1991) 'Coatomer': a cytosolic protein complex containing subunits of non-clathrin-coated Golgi transport vesicles. Nature **349:** 248–251
21 Waters M. G., Griff I. C. and Rothman J. E. (1991) Proteins involved in vesicular transport and membrane fusion. Curr. Opin. Cell Biol. **3:** 615–620
22 Serafini T., Orci L., Amherdt M., Brunner M., Kahn R. A. and Rothman J. E. (1991) ADP-ribosylation factor is a subunit of the coat of Golgi-derived COP-coated vesicles: a novel role for a GTP-binding protein. Cell **67:** 239–253
23 Donaldson J. G., Cassel D., Kahn R. A. and Klausner R. D. (1992) ADP-ribosylation factor, a small GTP-binding protein, is required for binding of the coatomer protein β-COP to Golgi membranes. Proc. Natl. Acad. Sci. USA **89:** 6408–6412
24 Aridor M., Bannykh S. I., Rowe T. and Balch W. E. (1995) Sequential coupling between CopII and CopI vesicle coats in endoplasmic reticulum to Golgi transport. J. Cell Biol. **131:** 875–893
25 Stephens D. J., Lin-Marq N., Pagano A., Pepperkok R. and Paccaud J. P. (2000) COPI-coated ER to Golgi transport complexes segregate from COPII in close proximity to ER exit sites. J. Cell Sci. **113:** 2177–2185
26 Ward T. H., Polishchuk R. S., Caplan S., Hirschberg K. and Lippincott-Schwartz J. (2001) Maintenance of Golgi structure and function depends on the integrity of ER export. J. Cell Biol. **155:** 557–570
27 Donaldson J. G., Finazzi D. and Klausner R. D. (1992) Brefeldin A inhibits Golgi membrane-catalysed exchange of guanine nucleotide onto ARF protein. Nature **360:** 350–352
28 Peyroche A., Antonny B., Robineau S., Acker J., Cherfils J. and Jackson C. L. (1999) Brefeldin A acts to stabilize an abortive ARF-GDP-Sec7 domain protein complex: involvement of specific residues of the Sec7 domain. Mol. Cell **3:** 275–285
29 Letourneur F., Gaynor E. C., Hennecke S., Demollière C., Duden R., Emr S. D. et al. (1994) Coatomer is essential for retrieval of dilysine-tagged proteins to the endoplasmic reticulum. Cell **79:** 1199–1207
30 Pelham H. R. B. (1994) About turn for the COPs? Cell **79:** 1125–1127
31 Rothman J. E. and Wieland F. T. (1996) Protein sorting by transport vesicles. Science **272:** 227–234
32 Lippincott-Schwartz J., Roberts T. H. and Hirschberg K. (2000) Secretory protein trafficking and organelle dynamics in living cells. Annu. Rev. Cell Dev. Biol. **16:** 557–589
33 Orci L., Amherdt M., Ravazzola M., Perrelet A. and Rothman J. E. (2000) Exclusion of Golgi residents from transport vesicles budding from Golgi cisternae in intact cells. J. Cell Biol. **150:** 1263–1270
34 Presley J. F., Ward T. H., Pfeifer A. C., Siggia E. D., Phair R. D. and Lippincott-Schwartz J. (2002) Dissection of COPI and Arf1 dynamics in vivo and role in Golgi membrane transport. Nature **417:** 187–193
35 Lowe M. and Kreis T. E. (1998) Regulation of membrane traffic in animal cells by COPI. Biochim. Biophys. Acta **1404:** 53–66
36 Clermont Y., Rambourg A. and Hermo L. (1994) Connections between the various elements of the *cis*- and mid-compartments of the Golgi apparatus of early rat spermatids. Anat. Rec. **240:** 469–480
37 Krijnse-Locker J., Ericsson M., Rottier P. J. and Griffiths G. (1994) Characterization of the budding compartment of

mouse hepatitis virus: evidence that transport from the RER to the Golgi complex requires only one vesicular transport step. J. Cell Biol. **124:** 55–70
38 Presley J. F., Cole N. B., Schroer T. A., Hirschberg K., Zaal K. J. M. and Lippincott-Schwartz J. (1997) ER-to-Golgi transport visualized in living cells. Nature **389:** 81–85
39 Scales S. J., Pepperkok R. and Kreis T. E. (1997) Visualization of ER-to-Golgi transport in living cells reveals a sequential mode of action for COPII and COPI. Cell **90:** 1137–1148
40 Allan B. B., Moyer B. D. and Balch W. E. (2000) Rab1 recruitment of p115 into a cis-SNARE complex: programming budding COPII vesicles for fusion. Science **289:** 444–448
41 Moyer B. D., Allan B. B. and Balch W. E. (2001) Rab1 interaction with a GM130 effector complex regulates COPII vesicle cis-Golgi tethering. Traffic **2:** 268–276
42 Tang B. L., Low S. H., Hauri H.-P. and Hong W. (1995) Segregation of ERGIC53 and the mammalian KDEL receptor upon exit from the 15 degrees C compartment. Eur. J. Cell Biol. **68:** 398–410
43 Klumperman J., Schweizer A., Clausen H., Tang B. L., Hong W., Oorschot V. et al. (1998) The recycling pathway of protein ERGIC-53 and dynamics of the ER-Golgi intermediate compartment. J. Cell Sci. **111:** 3411–3425
44 Lewis M. J. and Pelham H. R. B. (1992) Ligand-induced redistribution of a human KDEL receptor from the Golgi complex to the endoplasmic reticulum. Cell **68:** 353–364
45 Sciaky N., Presley J., Smith C., Zaal K. J. M., Cole N., Moreira J. E. et al. (1997) Golgi tubule traffic and the effects of brefeldin A visualized in living cells. J. Cell Biol. **139:** 1137–1155
46 Mironov A. Jr, Luini A. and Mironov A. (1998) A synthetic model of intra-Golgi traffic. FASEB J. **12:** 249–252
47 Pelham H. R. B. (2001) Traffic through the Golgi apparatus. J. Cell Biol. **155:** 1099–1101
48 Beznoussenko G. V. and Mironov A. A. (2002) Models of intracellular transport and evolution of the Golgi complex. Anat. Rec. **268:** 226–238
49 Glick B. S., Elston T. and Oster G. (1997) A cisternal maturation mechanism can explain the asymmetry of the Golgi stack. FEBS Lett. **414:** 177–181
50 Clermont Y., Xia L., Rambourg A., Turner J. D. and Hermo L. (1993) Transport of casein submicelles and formation of secretion granules in the Golgi apparatus of epithelial cells of the lactating mammary gland of the rat. Anat. Rec. **235:** 363–373
51 Mironov A. A., Weidman P. and Luini A. (1997) Variations on the intracellular transport theme: maturing cisternae and trafficking tubules. J. Cell Biol. **138:** 481–484
52 Glick B. S. and Malhotra V. (1998) The curious status of the Golgi apparatus. Cell **95:** 883–889
53 Lanoix J., Ouwendijk J., Lin C.-C., Stark A., Love H. D., Ostermann J. et al. (1999) GTP hydrolysis by arf-1 mediates sorting and concentration of Golgi resident enzymes into functional COP I vesicles. EMBO J. **18:** 4935–4948
54 Mironov A. A., Beznoussenko G. V., Nicoziani P., Martella O., Trucco A., Kweon H. S. et al. (2001) Small cargo proteins and large aggregates can traverse the Golgi by a common mechanism without leaving the lumen of cisternae. J. Cell Biol. **155:** 1225–1238
55 Rothman J. E. (1994) Mechanisms of intracellular protein transport. Nature **372:** 55–63
56 Pelham H. R. B. and Rothman J. E. (2000) The debate about transport in the Golgi – two sides of the same coin? Cell **102:** 713–719
57 Cosson P., Amherdt M., Rothman J. E. and Orci L. (2002) A resident Golgi protein is excluded from peri-Golgi vesicles in NRK cells. Proc. Natl. Acad. Sci. USA **99:** 12831–12834
58 Martínez-Menárguez J. A., Prekeris R., Oorschot V. M., Scheller R., Slot J. W., Geuze H. J. et al. (2001) Peri-Golgi vesicles contain retrograde but not anterograde proteins consistent with the cisternal progression model of intra-Golgi transport. J. Cell Biol. **155:** 1213–1224
59 Weidman P. (1995) Anterograde transport through the Golgi complex: do Golgi tubules hold the key? Trends Cell Biol. **5:** 302–305
60 Hirschberg K., Phair R. D., Polishchuk R. S., Gerlich D., Patterson G. H. and Lippincott-Schwartz J. (2002) Secretory transport from the Golgi is a mass action process and involves cargo-based membrane partitioning. Mol. Biol. Cell **13(S):** 1510
61 Hirschberg K., Miller C. M., Ellenberg J., Presley J. F., Siggia E. D., Phair R. D. et al. (1998) Kinetic analysis of secretory protein traffic and characterization of Golgi to plasma membrane transport intermediates in living cells. J. Cell Biol. **143:** 1485–1503
62 Toomre D., Keller P., White J., Olivo J. C. and Simons K. (1999) Dual-color visualization of trans-Golgi network to plasma membrane traffic along microtubules in living cells. J. Cell Sci. **112:** 21–33
63 Jones S. M., Howell K. E., Henley J. R., Cao H. and McNiven M. A. (1998) Role of dynamin in the formation of transport vesicles from the trans-Golgi network. Science **279:** 573– 577
64 Fricker M., Parson A., Tlalka M., Blancaflor E., Gilroy S., Meyer A. et al. (2001) Fluorescent probes for living cells. In: Plant Cell Biology, pp. 35–84, Hawes C. and Satiat-Jeunemaitre B. (eds), Oxford University Press, Oxford
65 Sheen J., Hwang S., Niwa Y., Kobayashi H. and Galbraith D. W. (1995) Green-fluorescent protein as a new vital marker in plant cells. Plant J. **8:** 777–784
66 Haseloff J., Siemering K. R., Prasher D. C. and Hodge S. (1997) Removal of a cryptic intron and subcellular localization of green fluorescent protein are required to mark transgenic *Arabidopsis* plants brightly. Proc. Natl. Acad. Sci. USA **94:** 2122–2127
67 Cruz S. S., Chapman S., Roberts A. G., Roberts I. M., Prior D. A. and Oparka K. J. (1996) Assembly and movement of a plant virus carrying a green fluorescent protein overcoat. Proc. Natl. Acad. Sci. USA **93:** 6286–6290
68 Köhler R. H. (1998) GFP for in vivo imaging of subcellular structures in plant cells. Trends Plant Sci. **3:** 317–320
69 Haseloff J. and Siemering K. R. (1998) The uses of GFP in plants. In: Green Fluorescent Protein: Strategies, Applications and Protocols, pp. 191–220, Chalfie M. and Kain S. (eds), Wiley, New York
70 Pelham H. R. B. (1990) The retention signal for soluble proteins of the endoplasmic reticulum. Trends Biochem. Sci. **15:** 483–486
71 Munro S. (1991) Sequences within and adjacent to the transmembrane segment of α-2,6-sialyltransferase specify Golgi retention. EMBO J. **10:** 3577–3588
72 Boevink P., Oparka K., Santa Cruz S., Martin B., Betteridge A. and Hawes C. (1998) Stacks on tracks: the plant Golgi apparatus traffics on an actin/ER network. Plant J. **15:** 441–447
73 Wee E. G.-T., Sherrier D. J., Prime T. A. and Dupree P. (1998) Targeting of active sialyltransferase to the plant Golgi apparatus. Plant Cell **10:** 1759–1768
74 Essl D., Dirnberger D., Gomord V., Strasser R., Faye L., Glössl J. et al. (1999) The N-terminal 77 amino acids from tobacco *N*-acetylglucosaminyltransferase I are sufficient to retain a reporter protein in the Golgi apparatus of *Nicotiana benthamiana* cells. FEBS Lett. **453:** 169–173
75 Nebenführ A., Gallagher L. A., Dunahay T. G., Frohlick J. A., Mazurkiewicz A. M., Meehl J. B. et al. (1999) Stop-and-go movements of plant Golgi stacks are mediated by the acto-myosin system. Plant Physiol. **121:** 1127–1141
76 Mitsuda N., Enami K., Nakata M., Takeyasu K. and Sato M. H. (2001) Novel type *Arabidopsis thaliana* H^+-PPase is localized to the Golgi apparatus. FEBS Lett. **488:** 29–33

77 Saint-Jore C. M., Evins J., Batoko H., Brandizzi F., Moore I. and Hawes C. (2002) Redistribution of membrane proteins between the Golgi apparatus and endoplasmic reticulum in plants is reversible and not dependent on cytoskeletal networks. Plant J. **29:** 661–678
78 Brandizzi F., Snapp E. L., Roberts A. G., Lippincott-Schwartz J. and Hawes C. (2002) Membrane protein transport between the endoplasmic reticulum and the Golgi in tobacco leaves is energy dependent but cytoskeleton independent: evidence from selective photobleaching. Plant Cell **14:** 1293–1309
79 Nilsson T. and Warren G. (1994) Retention and retrieval in the endoplasmic reticulum and the Golgi apparatus. Curr. Opin. Cell Biol. **6:** 517–521
80 Andreeva A. V., Kutuzov M. A., Evans D. E. and Hawes C. R. (1997) Rab-GDP dissociation inhibitor isoforms in *Arabidopsis thaliana.* J. Exp. Bot. **48:** 2109–2110
81 Pimpl P., Movafeghi A., Coughlan S., Denecke J., Hillmer S. and Robinson D. G. (2000) In situ localization and in vitro induction of plant COPI-coated vesicles. Plant Cell **12:** 2219–2236
82 Phillipson B. A., Pimpl P., daSilva L. L., Crofts A. J., Taylor J. P., Movafeghi A. et al. (2001) Secretory bulk flow of soluble proteins is efficient and COPII dependent. Plant Cell **13:** 2005–2020
83 Ritzenthaler C., Nebenführ A., Movafeghi A., Stussi-Garaud C., Behnia L., Pimpl P. et al. (2002) Reevaluation of the effects of Brefeldin A on plant cells using tobacco Bright Yellow 2 cells expressing Golgi-targeted green fluorescent protein and COPI antisera. Plant Cell **14:** 237–261
84 Andreeva A. V., Zheng H., Saint-Jore C. M., Kutuzov M. A., Evans D. E. and Hawes C. R. (2000) Organization of transport from endoplasmic reticulum to Golgi in higher plants. Biochem. Soc. Trans. **28:** 505–512
85 Batoko H., Zheng H.-Q., Hawes C. and Moore I. (2000) A Rab1 GTPase is required for transport between the endoplasmic reticulum and Golgi apparatus and for normal Golgi movement in plants. Plant Cell **12:** 2201–2218
86 Takeuchi M., Ueda T., Sato K., Abe H., Nagata T. and Nakano A. (2000) A dominant negative mutant of Sar1 GTPase inhibits protein transport from the endoplasmic reticulum to the Golgi apparatus in tobacco and *Arabidopsis* cultured cells. Plant J. **23:** 517–525
87 Takeuchi M., Ueda T., Yahara N. and Nakano A. (2002) Arf1 GTPase plays roles in the protein traffic between the endoplasmic reticulum and the Golgi apparatus in tobacco and *Arabidopsis* cultured cells. Plant J. **31:** 499–515
88 Lippincott-Schwartz J., Yuan L. C., Bonifacino J. S. and Klausner R. D. (1989) Rapid redistribution of Golgi proteins into the ER in cells treated with brefeldin A: evidence for membrane cycling from Golgi to ER. Cell **56:** 801–813
89 Ladha S., Mackie A. R., Harvey L. J., Clark D. C., Lea E. J., Brullemans M. et al. (1996) Lateral diffusion in planar lipid bilayers: a fluorescence recovery after photobleaching investigation of its modulation by lipid composition, cholesterol, or alamethicin content and divalent cations. Biophys. J. **71:** 1364–1373
90 Lippincott-Schwartz J., Snapp E. and Kenworthy A. (2001) Studying protein dynamics in living cells. Nat. Rev. Mol. Cell Biol. **2:** 444–456
91 Ellenberg J., Lippincott-Schwartz J. and Presley J. F. (1998) Two-color green fluorescent protein time-lapse imaging. Biotechniques **25:** 838–846
92 White J., Keller P. and Stelzer E. H. K. (2001) Spatial partitioning of secretory cargo from Golgi resident proteins in live cells. BMC Cell Biol. **2:** 19
93 Dunn G. A., Dobbie I. M., Monypenny J., Holt M. R. and Zicha D. (2002) Fluorescence localization after photobleaching (FLAP): a new method for studying protein dynamics in living cells. J. Microsc. **205:** 109–112
94 Nichols B. J., Kenworthy A. K., Polishchuk R. S., Lodge R., Roberts T. H., Hirschberg K. et al. (2001) Rapid cycling of lipid raft markers between the cell surface and Golgi complex. J. Cell Biol. **153:** 529–541
95 Cole N. B., Smith C. L., Sciaky N., Terasaki M., Edidin M. and Lippincott-Schwartz J. (1996) Diffusional mobility of Golgi proteins in membranes of living cells. Science **273:** 797–801
96 Lipsky N. G. and Pagano R. E. (1985) A vital stain for the Golgi apparatus. Science **228:** 745–747
97 van Meer G., Stelzer E. H. K., Wijnaendts-van-Resandt R. W. and Simons K. (1987) Sorting of sphingolipids in epithelial (Madin-Darby canine kidney) cells. J. Cell Biol. **105:** 1623–1635
98 Cooper M. S., Cornell-Bell A. H., Chernjavsky A., Dani J. W. and Smith S. J. (1990) Tubulovesicular processes emerge from trans-Golgi cisternae, extend along microtubules and interlink adjacent trans-Golgi elements into a reticulum. Cell **61:** 135–145
99 Storrie B., Pepperkok R., Stelzer E. H. K. and Kreis T. E. (1994) The intracellular mobility of a viral membrane glycoprotein measured by confocal microscope fluorescence recovery after photobleaching. J. Cell Sci. **107:** 1309–1319
100 Wier M. L. and Edidin M. (1986) Effects of cell density and extracellular matrix on the lateral diffusion of major histocompatibility antigens in cultured fibroblasts. J. Cell Biol. **103:** 215–222
101 Nilsson T., Hoe M. H., Slusarewicz P., Rabouille C., Watson R., Hunte F. et al. (1994) Kin recognition between *medial* Golgi enzymes in HeLa cells. EMBO J. **13:** 562–574
102 Munro S. (1995) An investigation of the role of transmembrane domains in Golgi protein retention. EMBO J. **14:** 4695–4704
103 Cole N. B., Ellenberg J., Song J., DiEuliis D. and Lippincott-Schwartz J. (1998) Retrograde transport of Golgi-localized proteins to the ER. J. Cell Biol. **140:** 1–15
104 Dunphy W. G. and Rothman J. E. (1985) Compartmental organization of the Golgi stack. Cell **42:** 13–21
105 Nilsson T., Slusarewicz P., Hoe M. H. and Warren G. (1993) Kin recognition. A model for the retention of Golgi enzymes. FEBS Lett. **330:** 1–4
106 Blum R., Pfeiffer F., Feick P., Nastainczyk W., Kohler B., Schäfer K. H. et al. (1999) Intracellular localization and in vivo trafficking of p24A and p23. J. Cell Sci. **112:** 537–548
107 Helms J. B. (1995) Role of heterotrimeric GTP binding proteins in vesicular protein transport: indications for both classical and alternative G protein cycles. FEBS Lett. **369:** 84–88
108 Melancon P., Glick B. S., Malhotra V., Weidman P. J., Serafini T., Gleason M. L. et al. (1987) Involvement of GTP-binding 'G' proteins in transport through the Golgi stack. Cell **51:** 1053–1062
109 Robinson M. S. and Kreis T. E. (1992) Recruitment of coat proteins onto Golgi membranes in intact and permeabilized cells: effects of brefeldin A and G protein activators. Cell **69:** 129–138
110 Zaal K. J. M., Smith C. L., Polishchuk R. S., Altan N., Cole N. B., Ellenberg J. et al. (1999) Golgi membranes are absorbed into and reemerge from the ER during mitosis. Cell **99:** 589–601
111 Lippincott-Schwartz J. and Zaal K. J. M. (2000) Cell cycle maintenance and biogenesis of the Golgi complex. Histochem. Cell Biol. **114:** 93–103
112 Shorter J. and Warren G. (2002) Golgi architecture and inheritance. Annu. Rev. Cell Dev. Biol. **18:** 379–420
113 Terasaki M. (2000) Dynamics of the endoplasmic reticulum and Golgi apparatus during early sea urchin development. Mol. Biol. Cell **11:** 897–914
114 Nakamura N., Rabouille C., Watson R., Nilsson T., Hui N., Slusarewicz P. et al. (1995) Characterization of a *cis*-Golgi matrix protein, GM130. J. Cell Biol. **131:** 1715–1726

115 Barr F. A., Puype M., Vandekerckhove J. and Warren G. (1997) GRASP65, a protein involved in the stacking of Golgi cisternae. Cell **91:** 253–262
116 Bomsel M. and Mostov K. (1992) Role of heterotrimeric G proteins in membrane traffic. Mol. Biol. Cell **3:** 1317–1328
117 Brown W. J., DeWald D. B., Emr S. D., Plutner H. and Balch W. E. (1995) Role for phosphatidylinositol 3-kinase in the sorting and transport of newly synthesized lysosomal enzymes in mammalian cells. J. Cell Biol. **130:** 781–96
118 Muñiz M., Alonso M., Hidalgo J. and Velasco A. (1996) A regulatory role for cAMP-dependent protein kinase in protein traffic along the exocytic route. J. Biol. Chem. **271:** 30935–30941
119 Fanger G. R., Johnson N. L. and Johnson G. L. (1997) MEK kinases are regulated by EGF and selectively interact with Rac/Cdc42. EMBO J. **16:** 4961–4972
120 Freyberg Z., Sweeney D., Siddhanta A., Bourgoin S., Frohman M. and Shields D. (2001) Intracellular localization of phospholipase D1 in mammalian cells. Mol. Biol. Cell **12:** 943–955
121 Robbins E. and Gonatas N. K. (1964) Histochemical and ultrastructural studies on HeLa cell cultures exposed to spindle inhibitors with special reference to the interphase cell. J. Histochem. Cytochem. **12:** 704–711
122 Turner J. R. and Tartakoff A. M. (1989) The response of the Golgi complex to microtubule alterations: the roles of metabolic energy and membrane traffic in Golgi complex organization. J. Cell Biol. **109:** 2081–2088
123 Thyberg J. and Moskalewski S. (1985) Microtubules and the organization of the Golgi complex. Exp. Cell Res. **159:** 1–16
124 Cole N. B., Sciaky N., Marotta A., Song J. and Lippincott-Schwartz J. (1996) Golgi dispersal during microtubule disruption: regeneration of Golgi stacks at peripheral endoplasmic reticulum exit sites. Mol. Biol. Cell **7:** 631–650
125 Storrie B., White J., Röttger S., Stelzer E. H. K., Suganuma T. and Nilsson T. (1998) Recycling of Golgi-resident glycosyltransferases through the ER reveals a novel pathway and provides an explanation for nocodazole-induced Golgi scattering. J. Cell Biol. **143:** 1505–1521
126 Muñiz M., Nuoffer C., Hauri H.-P. and Riezman H. (2000) The Emp24 complex recruits a specific cargo molecule into endoplasmic reticulum-derived vesicles. J. Cell Biol. **148:** 925–930
127 Dahm T., White J., Grill S., Füllekrug J. and Stelzer E. H. K. (2001) Quantitative ER ↔ Golgi transport kinetics and protein separation upon Golgi exit revealed by vesicular integral membrane protein 36 dynamics in live cells. Mol. Biol. Cell **12:** 1481–1498
128 Vasudevan C., Han W., Tan Y., Nie Y., Li D., Shome K. et al. (1998) The distribution and translocation of the G protein ADP-ribosylation factor 1 in live cells is determined by its GTPase activity. J. Cell Sci. **111:** 1277–1285
129 Barr F. A., Nakamura N. and Warren G. (1998) Mapping the interaction between GRASP65 and GM130, components of a protein complex involved in the stacking of Golgi cisternae. EMBO J. **17:** 3258–3268
130 Pelletier L., Jokitalo E. and Warren G. (2000) The effect of Golgi depletion on exocytic transport. Nat. Cell Biol. **2:** 840–846
131 Seemann J., Jokitalo E., Pypaert M. and Warren G. (2000) Matrix proteins can generate the higher order architecture of the Golgi apparatus. Nature **407:** 1022–1026
132 Jokitalo E., Cabrera-Poch N., Warren G. and Shima D. T. (2001) Golgi clusters and vesicles mediate mitotic inheritance independently of the endoplasmic reticulum. J. Cell Biol. **154:** 317–330
133 Prescott A. R., Farmaki T., Thomson C., James J., Paccaud J.-P., Tang B.-L. et al. (2001) Evidence for prebudding arrest of ER export in animal cell mitosis and its role in generating Golgi partitioning intermediates. Traffic **2:** 321–335
134 Pelletier L., Stern C. A., Pypaert M., Sheff D., Ngô H. M., Roper N. et al. (2002) Golgi biogenesis in *Toxoplasma gondii.* Nature **418:** 548–552
135 Lippincott-Schwartz J., Donaldson J. G., Schweizer A., Berger E. G., Hauri H. P., Yuan L. C. et al. (1990) Microtubule-dependent retrograde transport of proteins into the ER in the presence of brefeldin A suggests an ER recycling pathway. Cell **60:** 821–836
136 Tang B. L., Low S. H. and Hong W. (1995) Differential response of resident proteins and cycling proteins of the Golgi to brefeldin A. Eur. J. Cell Biol. **68:** 199–205
137 Hendricks L. C., McClanahan S. L., McCaffery M., Palade G. E. and Farquhar M. G. (1992) Golgi proteins persist in the tubulovesicular remnants found in Brefeldin A-treated pancreatic acinar cells. Eur. J. Cell Biol. **58:** 202–213
138 Orci L., Perrelet A., Ravazzola M., Wieland F. T., Schekman R. and Rothman J. E. (1993) 'BFA bodies': a subcompartment of the endoplasmic reticulum. Proc. Natl. Acad. Sci. USA **90:** 11089–11093
139 Miles S., McManus H., Forsten K. E. and Storrie B. (2001) Evidence that the entire Golgi apparatus cycles in interphase HeLa cells: sensitivity of Golgi matrix proteins to an ER exit block. J. Cell Biol. **155:** 543–555
140 Lu Z., Joseph D., Bugnard E., Zaal K. J. M. and Ralston E. (2001) Golgi complex reorganization during muscle differentiation: visualization in living cells and mechanism. Mol. Biol. Cell **12:** 795–808
141 Bonfanti L., Mironov A. A. Jr, Martínez-Menárguez J. A., Martella O., Fusella A., Baldassarre M. et al. (1998) Procollagen traverses the Golgi stack without leaving the lumen of cisternae: evidence for cisternal maturation. Cell **95:** 993–1003
142 Satiat-Jeunemaitre B. and Hawes C. (1994) G.A.T.T. (A General Agreement on Traffic and Transport) and Brefeldin A in plant cells. Plant Cell **6:** 463–467
143 Satiat-Jeunemaitre B., Cole L., Bourett T., Howard R. and Hawes C. (1996) Brefeldin A effects in plant and fungal cells: something new about vesicle trafficking? J. Microsc. **181:** 162–177
144 Robineau S., Chabre M. and Antonny B. (2000) Binding site of brefeldin A at the interface between the small G protein ADP-ribosylation factor 1 (ARF1) and the nucleotide-exchange factor Sec7 domain. Proc. Natl. Acad. Sci. USA **97:** 9913–9918
145 Patterson G. H. and Lippincott-Schwartz J. (2002) A photoactivatable GFP for selective photolabeling of proteins and cells. Science **297:** 1873–1877