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. 1A). Protein transport from the ER to the Golgi occurs via ER exit sites, also known...
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...