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Imaging Secretory Pathway Dynamics in Living Plant Cells

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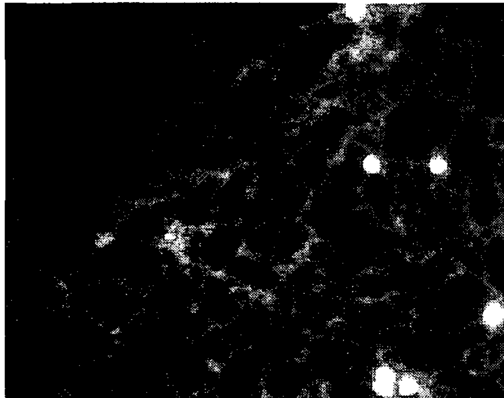
In the space of a decade the green fluorescent protein (GFP) has emerged as one of the most popular tools used by plant biologists for the study of dynamic cellular processes *in vivo*. Expression of the protein has revolutionised the scientific approach for the study of organelles in the secretory pathway, such as the Golgi apparatus and the endoplasmic reticulum (ER). As Golgi and ER proteins can be fused to GFP or its spectral derivatives, usually without altering their targeting and function, this is the preferred alternative to conventional



dyes which were used in the past to investigate these compartments *in vivo*. By the expression of ER (signal sequence-GFP-HDEL) and a number of Golgitargeted GFP constructs in tobacco and arabidopsis leaves, and in suspension culture cells (Fig. 1), we have shown that the plant Golgi apparatus is a highly dynamic organelle. Unlike the relatively static mammalian Golgi complex, plant Golgi bodies, at least in the cortex of vacuolate leaf cells, appear to be closely associated with the tubules of endoplasmic reticulum and appear to move over the surface of the ER. Such movement is dependent on an actin network, which underlies the cortical ER tubules, and can by analysed with particle tracking software. It thus appears that the Golgi are intimately associated with, if not directly connected to, the ER. Transport of protein between the ER and Golgi apparatus has been studied using fluorescence recovery after photobleaching (FRAP) fluorescent protein labelled Golgi. Targeting of both YFP and GFP to the same Golgi permits selective photobleaching of one protein whilst still imaging the organelle using the second protein. In this way transport of Golgi targeted constructs has been analysed between the ER and moving Golgi showing that fluorescence recovery and thus transport between ER and Golgi can occur in moving Golgi stacks. In yeast and mammalian cells it has been proposed that export of material to the Golgi is from specific export sites that are located on the ER and which depend on theformation of a protein coat the so-called COPII complex. This may then package cargo into COPII vesicles or tubular/vesicular carriers for transport to the Golgi. Plants possess homologues of the majority of the genes encoding COPII proteins. By expressing GFP constructs of the small GTPase Sarlp, which initiates COPII coat formation, and its non-functional mutants, we have established a role for the protein in regulating protein transport out of the ER. Using a mutant GTP-locked for of Sarlp we have identified exit sites on the plant ER which appear to be closely associated with individual Golgi bodies and appear to move over the surface of the ER in association with the Golgi bodies. Thus we propose that the Golgi and export sites together form a motile export complexes associated with the moving ER. By expressing the trans-membrane domains of the ER membrane protein calnexin fused to photoactivatable GFP and tracking UV activated protein, it can be shown that the surface of the ER moves in the same direction as the Golgi bodies. Finally, we have cloned a number of proteinsthat may form the basis of the plant Golgi matrix and act as tethers between the cisternae of the Golgi stacks. Fluorescent protein constructs of these locate to the Golgi and initial experiments indicate that over-expression results in disturbance of Golgi morphology.