

## Redistribution of a Golgi glycoprotein in plant cells treated with Brefeldin A

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### Summary

The fungal fatty acid derivative Brefeldin A (BFA), has been used to study the reversible distribution of a Golgi glycoprotein, the JIM 84 epitope, into the cytosol of higher plant cells. Treatment of both maize and onion root tip cells resulted in a rearrangement of the Golgi stacks into either circular formations or a perinuclear distribution. The Golgi cisternae became curved and vesiculated and in cells where the Golgi apparatus was totally dispersed the JIM 84 epitope was associated with

large areas in the cytosol which were also vesiculated. On removal of the BFA the Golgi apparatus reformed and the JIM 84 epitope was again located in the cisternal stacks. This mode of BFA action is compared with that so far described for animal cells.

Key words: Golgi apparatus, monoclonal antibody, JIM 84, Brefeldin A, vesicle traffic, endomembranes, exocytosis.

### Introduction

The plant cell secretory process is a result of sequential activity along a vesicle-mediated pathway from the endoplasmic reticulum (ER) to the cell surface (reviewed by Hawes et al., 1991). It is assumed that most of the proteins passing through the endomembrane system are transported by transition vesicles from the ER to the *cis* face of the Golgi stacks. They are then subject to modification and further glycosylation in the lumen of the Golgi cisternae before being packaged into vesicles again and transported to the cell surface along a default pathway (Denecke et al., 1990) or targeted to other cellular compartments (Chrispeels, 1991; Saalbach et al., 1991; Bednarek and Raikhel, 1991). In the majority of plant cells, however, the principal secretory products are carbohydrate in nature and enter the secretory pathway at the Golgi apparatus. Most cell wall polysaccharide precursors are constructed and processed in the Golgi stacks before being released in vesicles destined for the cell surface, and not necessarily just from the *trans*-Golgi network (Brummel et al., 1990; Moore et al., 1991; Staehelin et al., 1991; Lainé et al., 1991). The final maturation of both polysaccharides, proteins and glycoproteins may in fact take place in the secretory vesicles before they are released at the cell surface, e.g. fucosylation of xyloglucans (Brummel et al., 1990) or methylation of polygalacturonans (Vian and Roland, 1991), or in their vacuolar stor-

age compartment, e.g. sporamin (Matsuoka and Nakamura, 1991) or phytohemagglutinin (Höfte et al., 1991).

Thus, a picture of the Golgi apparatus as the central processing/targeting compartment of the plant cell is slowly being constructed (Harris and Watson, 1991). However, there are still many questions to be answered as to the exact organisation of functional pathways arriving at, or derived from the Golgi apparatus. Transport from the ER to the Golgi still remains a mystery, although it has recently been shown that the tetrapeptide (His-Asp-Glu-Leu), or HDEL, is an ER retention signal operative in plants (Fontes et al., 1991; Napier et al., 1992), which implies that a vesicle recycling system may operate from the *cis*-Golgi back to the ER (Pelham, 1991b). Data on transport from the Golgi to the vacuole have shown that several vacuolar targeting signals may operate in plants. However they appear to differ in position and sequence (Bednarek and Raikhel, 1991; Neuhaus et al., 1991; Saalbach et al., 1991; Chrispeels and Raikhel, 1992), and nothing is known about the interaction of the targeting sequences with putative receptors on the vesicle membrane. Likewise, there is some evidence for a flow of endocytosed material to the *trans*-Golgi network (Fowke et al., 1991) but nothing is yet known of the physiological importance of this pathway.

One approach to the study of the Golgi apparatus is to use drug-induced modification of the organelle. Using this strategy, Craig and Goodchild (1984a) demonstrated a

change in the targeting of vicilin from the vacuole to plasma membrane in monensin-treated cells. However, more recently a fungal fatty acid derivative, Brefeldin A (BFA), has been shown to be a potent agent in the disruption of the Golgi apparatus in animal cells (Fujiwara et al., 1988; Doms et al., 1989; Lippincott-Schwartz et al., 1989, 1990) causing a reversible redistribution of Golgi proteins into the ER along with a reabsorption of the Golgi apparatus into the ER.

We have recently demonstrated that BFA has a profound effect on the organisation of the Golgi apparatus in plant cells, and this is also reversible (Satiat-Jeunemaitre and Hawes, 1992), thus giving the plant cell biologist an important tool with which to study Golgi structure and function. We have also previously reported on the production of a monoclonal antibody, JIM 84, which is specific to the plant Golgi apparatus and can be used as an immunocytochemical marker for the Golgi (Horsley et al., 1993). In this paper we report on the redistribution of the JIM 84 epitope following BFA treatment and the concomitant re-establishment of the Golgi after BFA removal.

## Materials and methods

### Plant material

Maize seeds (var LG11, Limagrain) and onion seeds (White Lisbon) were grown on damp filter paper in the dark at room temperature. Root tips were excised from 4-day-old seedlings.

### Monoclonal antibody

The monoclonal antibody, JIM 84, was raised against a carrot cell coated-vesicle preparation, as previously reported (Horsley et al., 1993), and was used as undiluted culture supernatant.

### Brefeldin A treatment

Brefeldin A (BFA) (Cambio, England, or Tebu, France) was used within a range of concentrations from 50 to 200 µg/ml. Roots from intact 4-day-old seedlings were immersed in aqueous BFA solutions for 3 h. Controls were made by immersion in water. For reversibility experiments, roots were carefully washed, and left to recover for 1-3 h in distilled water before fixation for microscopy.

### Immunofluorescence

All of the following steps were carried out at room temperature. Maize and onion root tips were excised in 2-3 mm segments under 4% paraformaldehyde in 0.1 M PIPES buffer, pH 6.9 and fixed for 1 h. After thorough washing root tips were incubated in 2% Cellulysin (Calbiochem) for 40 min to permeabilise the cell walls. After washing in buffer, root tips were squashed onto poly-L-lysine-coated coverslips to release individual cells. The preparations were then left to dry. Cells were further permeabilised with 0.5% Triton X-100 in buffer for 5 min. Preparations were incubated in 1% bovine serum albumin in PIPES buffer for 5 min and then incubated in JIM 84 for up to 1 h. Washings were performed for 1 h with PIPES buffer containing 0.1% fish gelatin, washed thoroughly and stained for 1 h with an FITC anti-rat secondary antibody (Serotec, Kidlington, UK), diluted 1:40 in buffer. After a thorough washing, preparations were mounted in Citifluor antifade (City University, London, UK) and observed with a Zeiss epifluorescence microscope with FITC filter set. Micrographs were recorded on Ilford HP5 film at 400 ASA.

### Electron microscopy

For conventional observations, root tips were prepared and embedded in Spurr resin as follows: roots were fixed in 1% paraformaldehyde and 2% glutaraldehyde in sodium cacodylate buffer (0.1 M, pH 6.8) for 1 h, washed thoroughly and soaked in 1% tannic acid for 1 h. Post-fixation was for 1 h in 1% aqueous OsO<sub>4</sub>. After washing in distilled water, specimens were stained *en bloc* with 0.5% uranyl acetate overnight at 4°C. Specimens were dehydrated in a graded water/ethanol series and embedded in Spurr resin. Sections were cut with a Reichert Ultracut E ultramicrotome and collected on nickel or copper grids and stained with lead citrate.

For immunogold labelling, root tips were fixed in 1% paraformaldehyde and 1% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 6.9) for 1 h at room temperature. Specimens were then dehydrated in a graded water/ethanol series and low temperature-embedded in LR White resin with 1% benzoin methyl ether, using a modified method of VandenBosh (1991) as follows: 10% EtOH, 20°C, 10 min; 20% EtOH, 20°C, 10 min; 30% EtOH, 0°C, 1 h; 50% EtOH, -20°C, 1 h; 75% EtOH, -20°C, 1 h; 90% EtOH, -20°C, 1 h; 100% EtOH, -20°C, 1 h; absolute EtOH, -20°C, 1 h; ethanol/resin mixtures of 2:1, 1:1, 1:2, by volume, -20°C, for 1 h each; four baths of pure resin, -20°C, for 24 h each. The resin was polymerised with UV light for 24 h at -20°C, and then 16 h at 15°C in gelatin capsules or plastic beads.

Sections were cut on a Reichert Ultracut E Ultramicrotome, collected on nickel or gold grids and stained by flotation on droplets of reagent as follows: 10 min in Tris-buffered saline plus 1% globulin-free BSA (TBSB) and 0.1% Tween 20, 10 min wash in TBSB, 1 h in JIM 84 (neat supernatant), 1 h wash in TBSB with 4 changes, 1 h in 10 nm gold-conjugated goat anti-rat IgM (BioCell, Cardiff) diluted 1:20 with the buffer, 4 washes in TBSB and 2 washes with ultrapure water. Sections were post-stained with 2% aqueous uranyl acetate (3 min), sometimes followed by lead citrate (2 min).

All sections were examined with a JEOL 1200 EXII transmission electron microscope, at 100 kV.

## Results

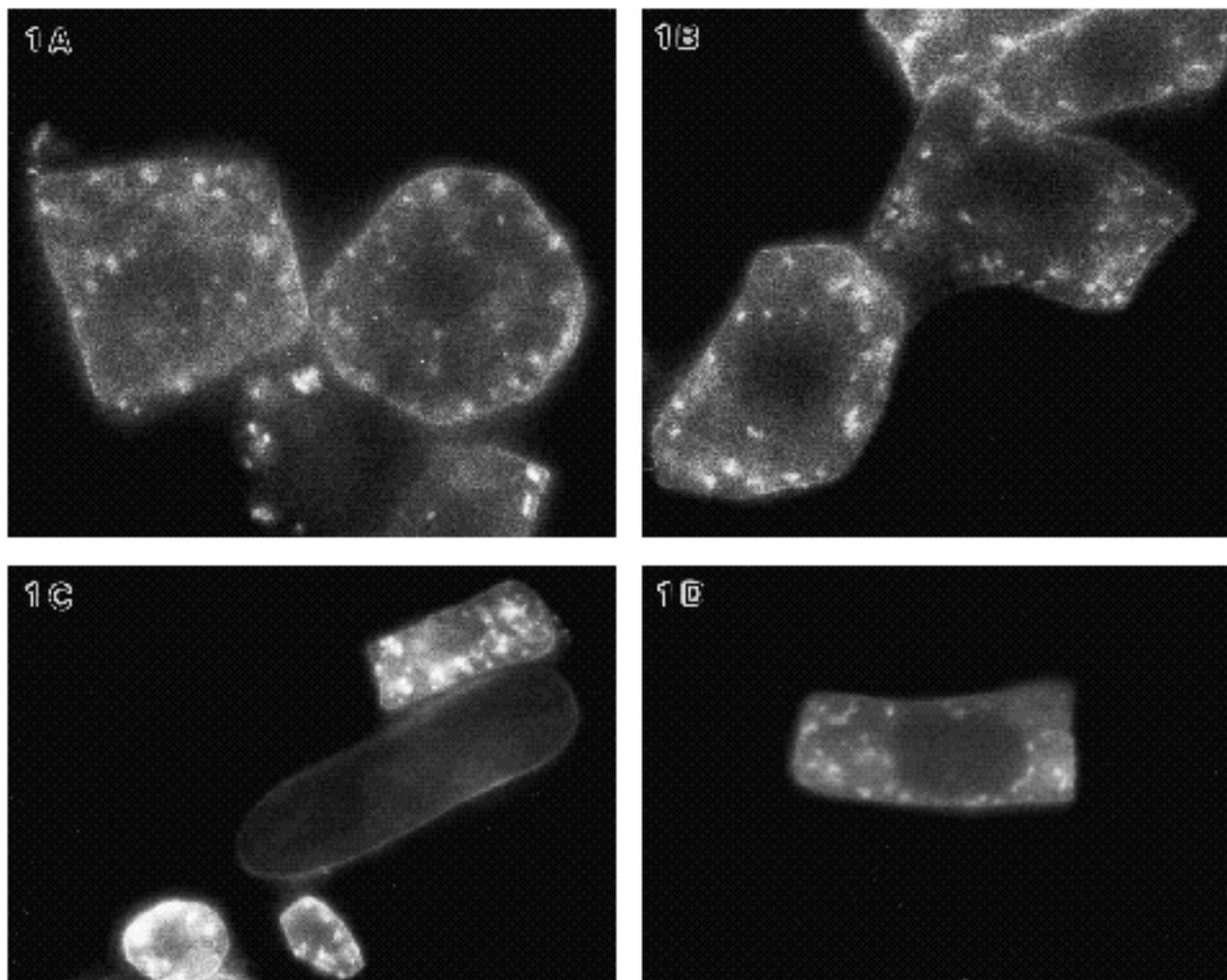
### Light microscopy

#### Control cells

Immunofluorescence microscopy of onion and maize root tip cells stained with JIM 84 reveals similar labelling patterns (Fig. 1). Fluorescent staining of discrete organelles is observed in the cells. These fluorescent organelles are uniformly distributed throughout the cortical and perinuclear cytoplasm (Fig. 1A,B,D). The labelling is observed in most cell types, although fully differentiated mucilage-secreting root cap cells of maize do not stain (Fig. 1C). Immunogold localisation by electron microscopy confirms that these organelles are the Golgi stacks both in maize (Fig. 5A,B) and onion cells (Fig. 5D,E). In most cases, the plasma membrane is also stained, suggesting that the epitope recognised by JIM 84 antibody is associated with both the Golgi apparatus and the plasma membrane in these root cells.

#### BFA-treated cells

BFA-induced modifications in the labelling pattern of root cells are clearly observed at concentrations of 50 µg/ml, 100 µg/ml and 200 µg/ml BFA in the external medium. However, there are slight variations in intensity of response



**Fig. 1.** Localisation of the JIM 84 epitope in root cells by immunofluorescence. (A, B) Maize root cells showing Golgi staining throughout the cytoplasm (cortical and perinuclear). In most cases, the plasma membranes are also stained. (A,  $\times 1\ 360$ ; B,  $\times 1\ 480$ ). (C) Differentiated maize root cap cells are not stained ( $\times 760$ ). (D) Staining of onion root cell reveals a similar immunofluorescence pattern ( $\times 1\ 200$ ).

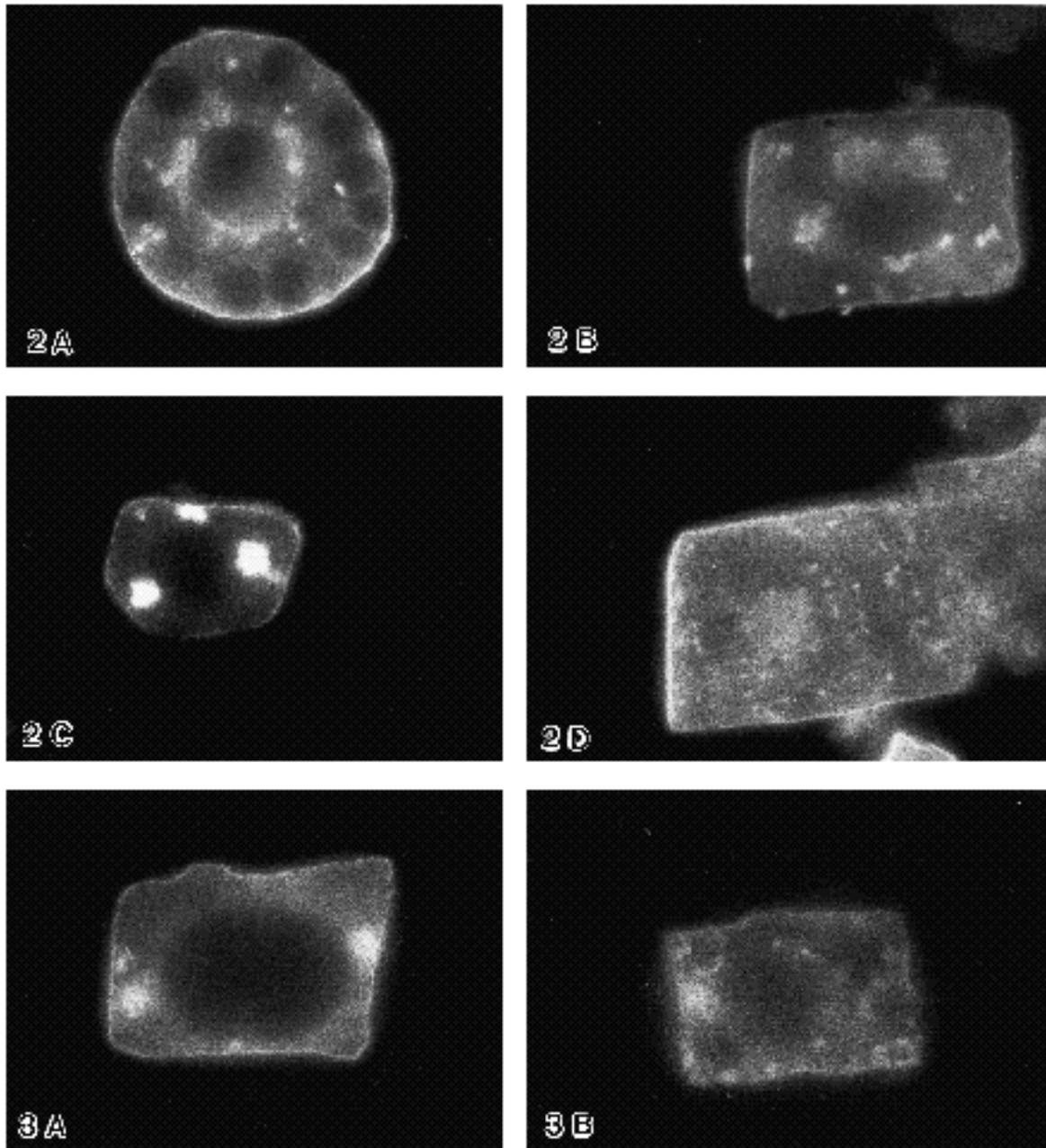
to BFA treatment from one cell to another in the same root, and from one experiment to another. Therefore, it appears obvious that the sensitivity to BFA (and therefore the intensity of the BFA-induced response) varies according either to the root, or to the cell size or type observed. Because of the occurrence of these internal variations in the BFA response, no clear differences can be attributed to the results obtained from onion and maize roots. Consequently, from this initial investigation, BFA-induced modifications appear similar in maize and onion root cells and will be considered together.

**50  $\mu\text{g/ml}$  BFA treatment.** Striking effects of BFA on the distribution of the JIM 84 epitope are apparent after a 50  $\mu\text{g/ml}$  BFA treatment both in maize (Fig. 2) and onion (Fig. 3) root cells. Instead of the characteristic fluorescent spots uniformly distributed in the cytosol as observed in the control cells (Fig. 1), the staining is restricted to discrete cytoplasmic areas. The staining patterns differ slightly from one cell type to another. In some cells the Golgi stacks are still

identifiable, grouped together around the perinuclear area (Fig. 2A); they can also be held together in a circular pattern in the cytoplasm, again often concentrated in the perinuclear region (Fig. 2B; see also Fig. 4A,B,C where these features are more pronounced in 200  $\mu\text{g/ml}$  BFA-treated cells). In some other cells, the fluorescence is brightly concentrated in three-to-four circular zones in the cytoplasm and the Golgi stacks are no longer discernable (Fig. 2C for maize cells, Fig. 3A for onion cells). The staining of the plasma membrane observed in control cells remains after a 50  $\mu\text{g/ml}$  BFA treatment.

After a recovery treatment in water the characteristic Golgi pattern displayed by the JIM 84 staining returns in both maize cells (Fig. 2D) and onion cells (Fig. 3B). Therefore, it is clear that the BFA effects on the subcellular location of the JIM 84 epitope are reversible.

**200  $\mu\text{g/ml}$  BFA treatment.** Following a 200  $\mu\text{g/ml}$  BFA treatment, root cells commonly display typical perinuclear areas of fluorescence, as already described in 50  $\mu\text{g/ml}$  BFA



**Fig. 2.** Modification of the fluorescence pattern in maize root cells after incubation in 50  $\mu\text{g/ml}$  BFA, and recovery. (A) Golgi staining is mainly localised around the nucleus ( $\times 980$ ). (B) Fluorescence often concentrates in discrete circular compartments in the perinuclear cytoplasm ( $\times 1\ 120$ ). (C) Some cells exhibit highly fluorescent compartments where discrete organelles are no longer visible ( $\times 1\ 320$ ). (D) Reappearance of the staining of discrete organelles throughout the cytoplasm following a 3 h recovery in water after 50  $\mu\text{g/ml}$  BFA ( $\times 1\ 560$ ).

**Fig. 3.** Modifications of the fluorescence pattern in onion root cells after incubation in 50  $\mu\text{g/ml}$  BFA and recovery process. (A) Concentration of fluorescence in circular areas in the cytosol of treated cells ( $\times 1\ 440$ ). (B) Reappearance of individual Golgi staining following a 3 h recovery ( $\times 1\ 520$ ).

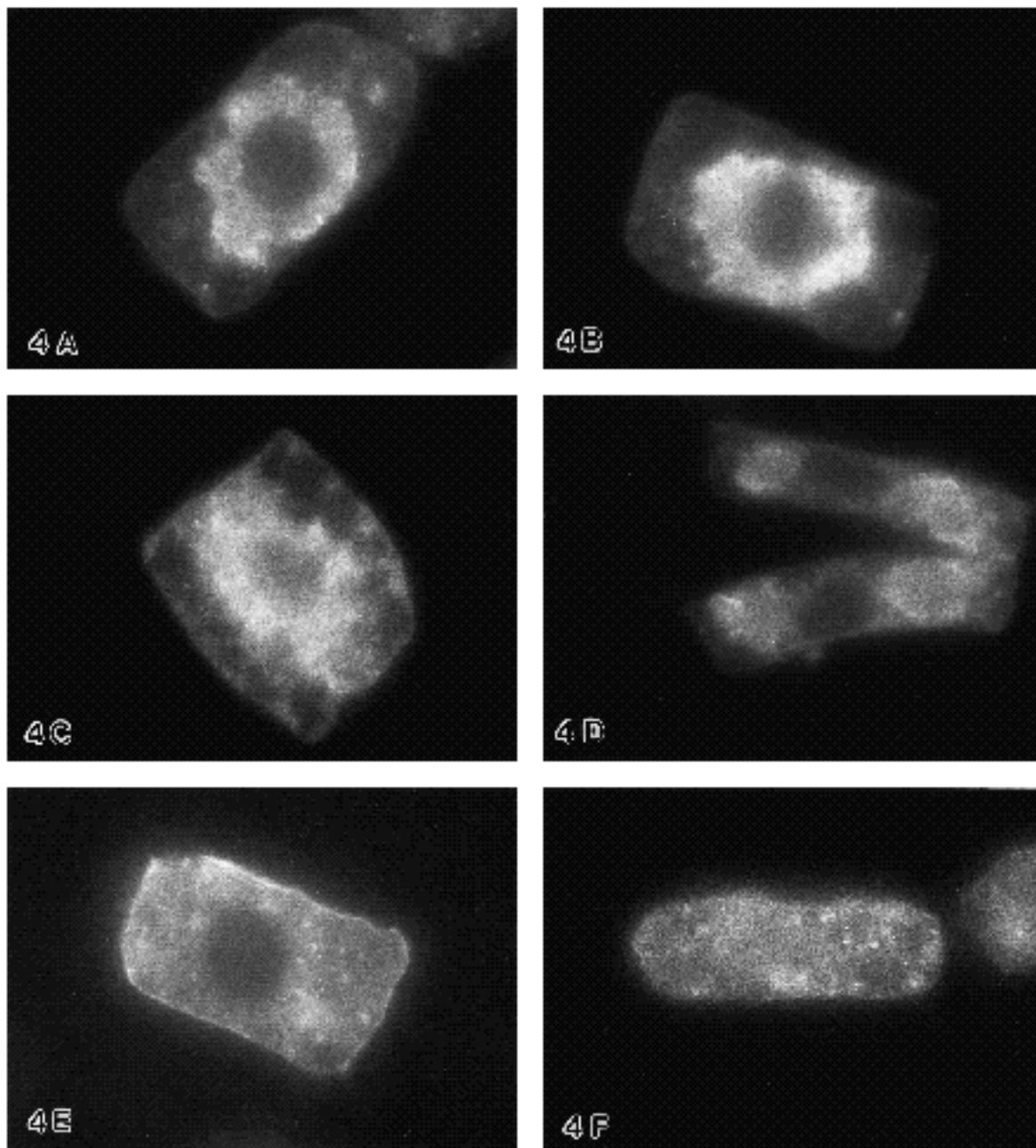
treatments (Fig. 4A,B,C). In elongated cells, two circular areas are often found, on either side of the nucleus (Fig. 4D). In most cases, the periphery of these areas is more fluorescent than the center. At this concentration of BFA, plasma membrane labelling is lost or reduced (Fig. 4A-D). After a recovery treatment, the BFA effects are reversed and the typical Golgi labelling pattern is restored (Fig.

4E,F). Interestingly, in two experiments the number of Golgi stacks after a recovery treatment appeared greater than in control cells (not shown).

#### *Electron microscopy*

##### *Control cells*

In untreated cells, immunogold labelling of root sections



**Fig. 4.** Modification of the fluorescent pattern following a 200  $\mu\text{g/ml}$  BFA treatment in maize root cells and recovery process. (A, B, C) Intense fluorescence localised in the perinuclear region, comprising several circular areas ( $\times 1120$ ). (D) Two circular areas of staining either side of the nucleus in elongate cells ( $\times 1080$ ). (E, F) Following a recovery treatment, the JIM 84 epitope is redistributed back into discrete cytoplasmic organelles. (E,  $\times 1120$ ; F,  $\times 1160$ ).

after incubation with JIM 84 demonstrates the high affinity of the antibody for the Golgi apparatus (Fig. 5A,B,D,E) in both maize and onion cells. Gold particles appear distributed over all the cisternae of the Golgi apparatus in all cells observed. In general, the number of gold particles per Golgi stack varies from 15 to 60, and rarely falls below 15. In both onion and maize root cells, the plasma membrane generally displays a patchy labelling, often associated with the outer surface of the plasma membrane (Fig. 5C,F). The intensity and the regularity of this staining varies from one

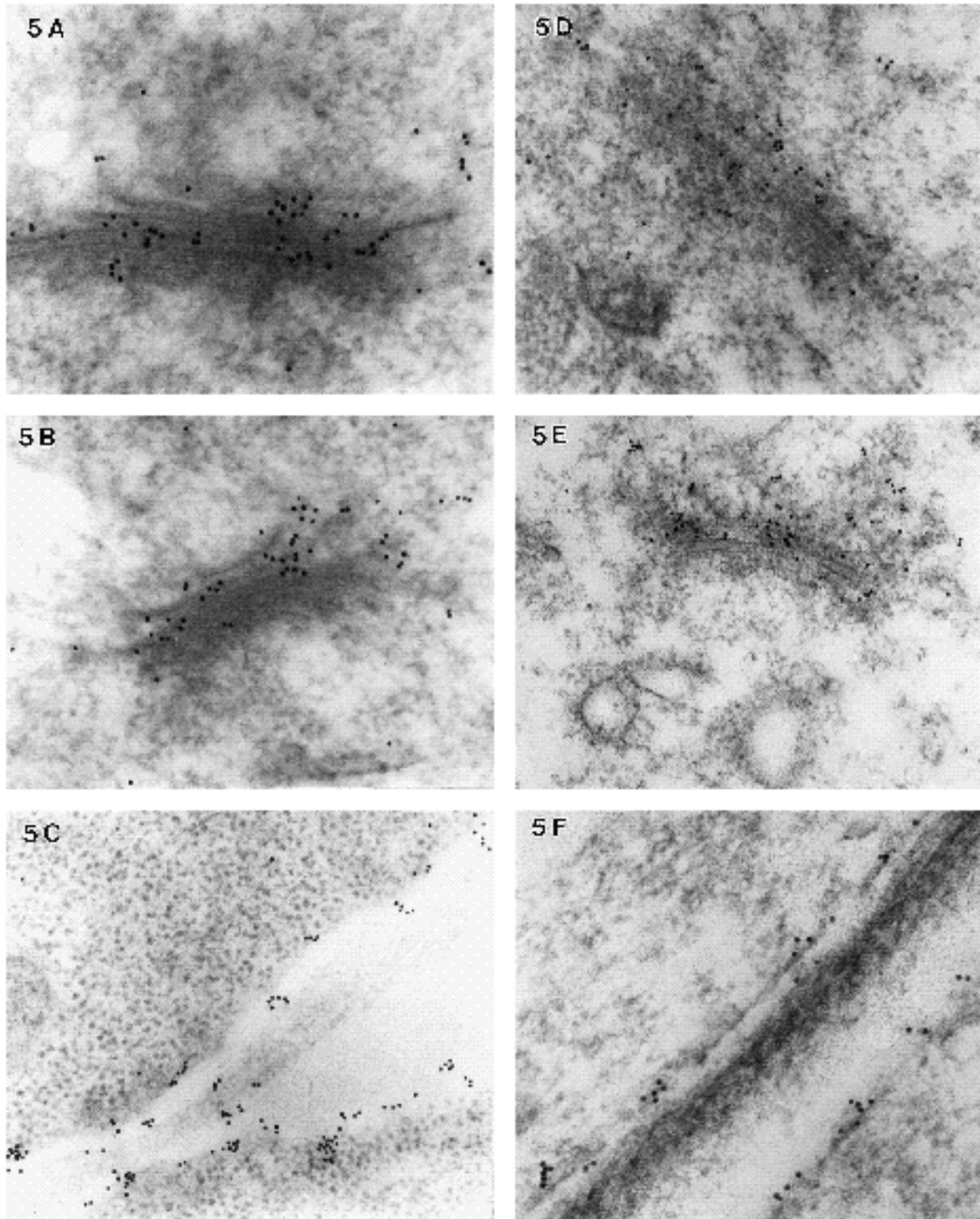
cell to another, from low levels (i.e. differentiated root cap), to a more continuous gold coating over the plasma membrane. Specific staining was not observed in other organelles.

#### *BFA-treated cells*

By electron microscopy it is also apparent that cells show varying responsiveness to BFA treatment. A few cells treated with 50  $\mu\text{g/ml}$  BFA show the same extensive Golgi modification as seen in most 200  $\mu\text{g/ml}$  treated cells. Con-

versely, in some 200  $\mu\text{g/ml}$  BFA-treated cells occasional Golgi stacks can be observed, which display a JIM 84

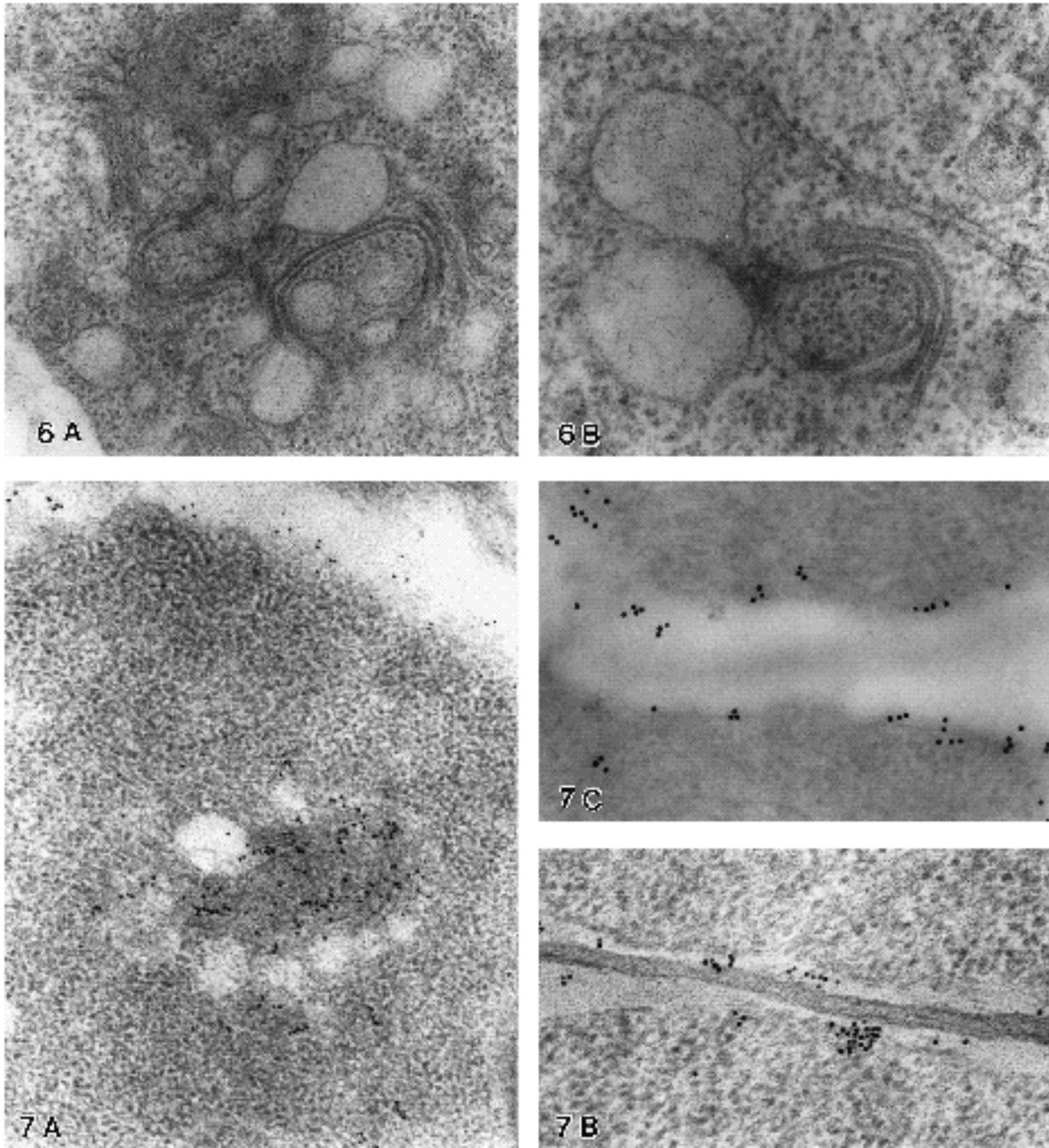
labelling similar to those in the control and in some of the 50  $\mu\text{g/ml}$  BFA treated cells.



**Fig. 5.** Localisation of the JIM 84 epitope by immunogold labelling on ultrathin sections of root cells. (A, B) Golgi staining in maize root cells. Gold particles are localised over all the cisternae (A,  $\times 117\ 000$ ; B,  $\times 88\ 000$ ). (C) Plasma membranes exhibit patches of gold particles mostly on the outer side of the membranes ( $\times 80\ 000$ ). (D, E) Golgi staining in onion root cells. Again no preferential staining of a particular Golgi cisternae can be detected (D,  $\times 88\ 200$ ; E,  $\times 66\ 500$ ). (F) Plasma membrane staining in onion root cells ( $\times 116\ 000$ ).

*BFA-induced changes in Golgi morphology.* Following BFA treatment, the Golgi apparatus is disrupted as has previously been described for plant cells (Satiat-Jeunemaitre and Hawes, 1992). With 50  $\mu\text{g/ml}$  BFA, Golgi cisternae tend to be curved, especially at the *trans* face, and numerous vesicles or vacuoles appear around the stacks. At higher

BFA concentrations the number of Golgi cisternae in individual stacks decreases, and the vesiculation process is enhanced (Fig. 6A,B). With 200  $\mu\text{g/ml}$  BFA, the total number of Golgi stacks per cell is greatly reduced, and in many cells individual Golgi stacks are no longer recognisable.

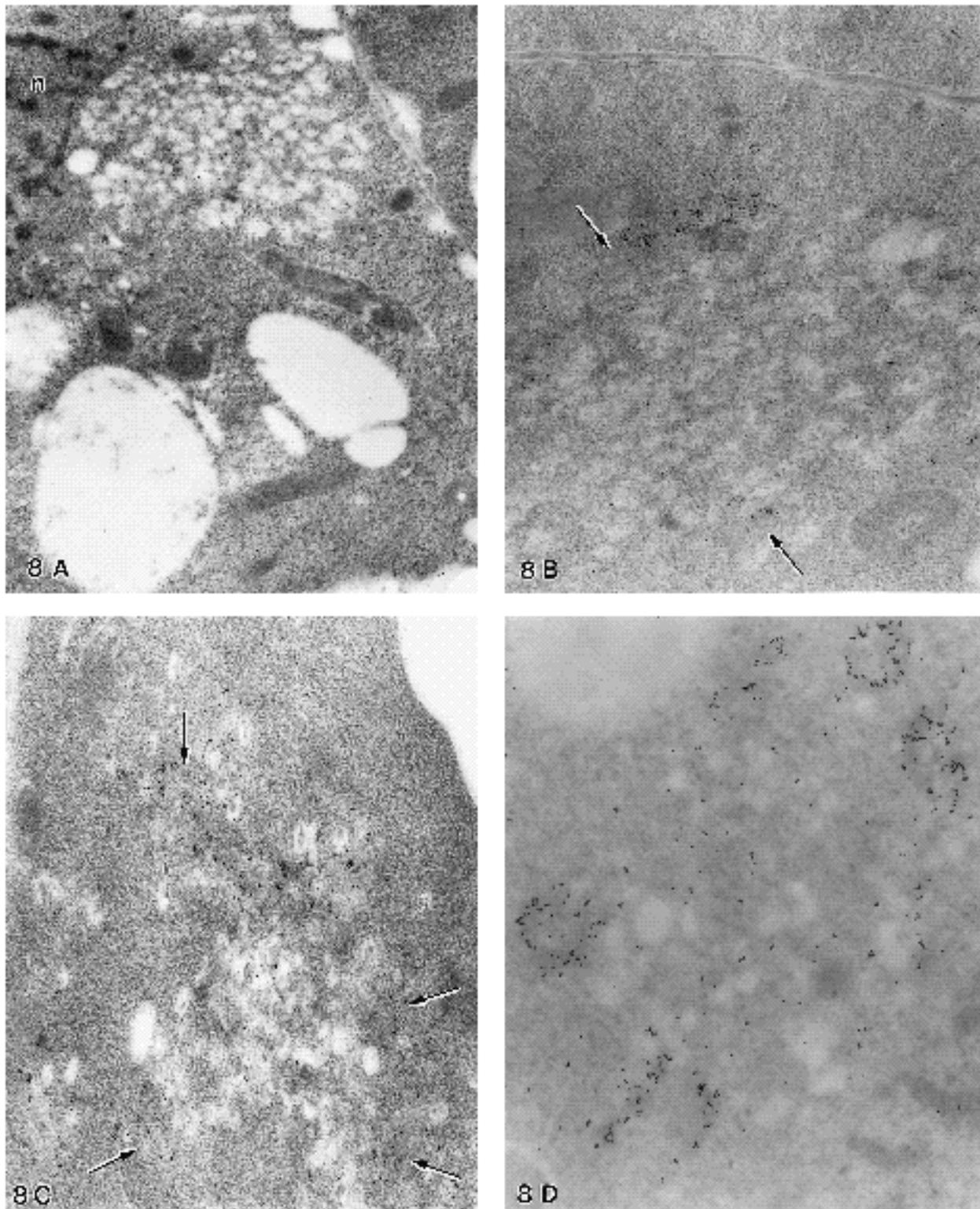


**Fig. 6.** Spurr embedded ultrathin section of 100  $\mu\text{g/ml}$  BFA treated maize root cells. (A, B) Reduction in the number of cisternae in Golgi stacks. The Golgi cisternae appear abnormally curved and numerous large vesicles are apparent (A,  $\times 45\ 000$ ; B,  $\times 64\ 000$ ).

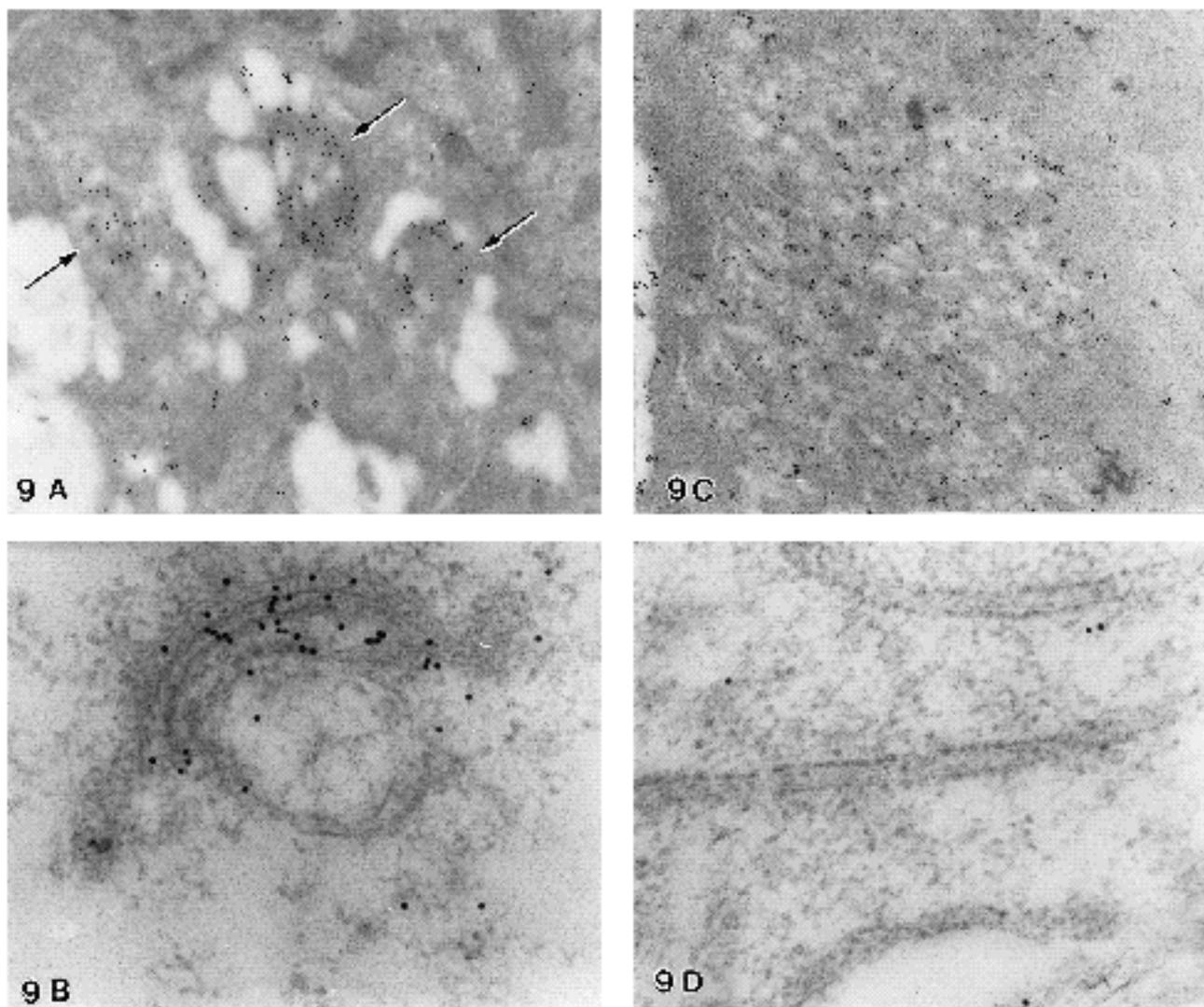
**Fig. 7.** Localisation of the JIM 84 epitope in 50  $\mu\text{g/ml}$  BFA-treated maize root cells. (A) Immunogold labelling on LRWhite ultrathin sections: gold labelling of the perturbed Golgi stack but not the associated large vesicles is visible ( $\times 63\ 500$ ). (B, C) Staining of the plasma membrane is still apparent after a 50  $\mu\text{g/ml}$  BFA treatment (B), or a 200  $\mu\text{g/ml}$  BFA treatment (C). (B,  $\times 91\ 500$ ; C,  $\times 95\ 000$ ).

*Redistribution of the JIM 84 antigen.* The characteristic appearance of curved Golgi stacks after BFA treatment is

commonly observed in LR white sections of 50  $\mu\text{g/ml}$  BFA-treated root cells (Fig. 7A) and on some 200  $\mu\text{g/ml}$  BFA-



**Fig. 8.** Localisation of the JIM 84 epitope in 200  $\mu\text{g/ml}$  BFA-treated maize root cells. (A) Formation of large vesiculated areas of cytoplasm, where gold particles are preferentially found, near the nucleus (n) ( $\times 16\,000$ ). (B, C) The remains of Golgi membranes after the BFA treatment are often seen at the periphery of the vesiculated areas, encircling the area (arrows). Gold particles are also inside the vesiculated area (B,  $\times 35\,000$ ; C,  $\times 33\,500$ ). (D) Lower contrast section showing several gold patches around a vesiculated areas; the patches reflect the presence of the BFA-perturbed Golgi stacks. Note also gold particles inside the vesiculated area. ( $\times 32\,500$ ).



**Fig. 9** Immunogold localisation of JIM 84 epitope in onion root cells following a BFA treatment. (A) 200  $\mu\text{g/ml}$  BFA. Gold particles are preferentially associated with large vesiculated circular, and label curved membranes at the periphery of the area (arrows) ( $\times 32\,500$ ). (B) Higher magnification showing a specific labelling of the BFA-disturbed Golgi stacks ( $\times 107\,500$ ). (C) 50  $\mu\text{g/ml}$  BFA. Gold particles label the whole vesiculated area. Note the labelling of the plasma membrane (left hand side of the micrograph) and the gold particles-free cytoplasm outside the vesiculated area (right side of the micrograph) ( $\times 33\,500$ ). (D) Endoplasmic reticulum in 200  $\mu\text{g/ml}$  BFA-treated cells. Note total lack of immunolabelling with JIM 84 ( $\times 95\,500$ ).

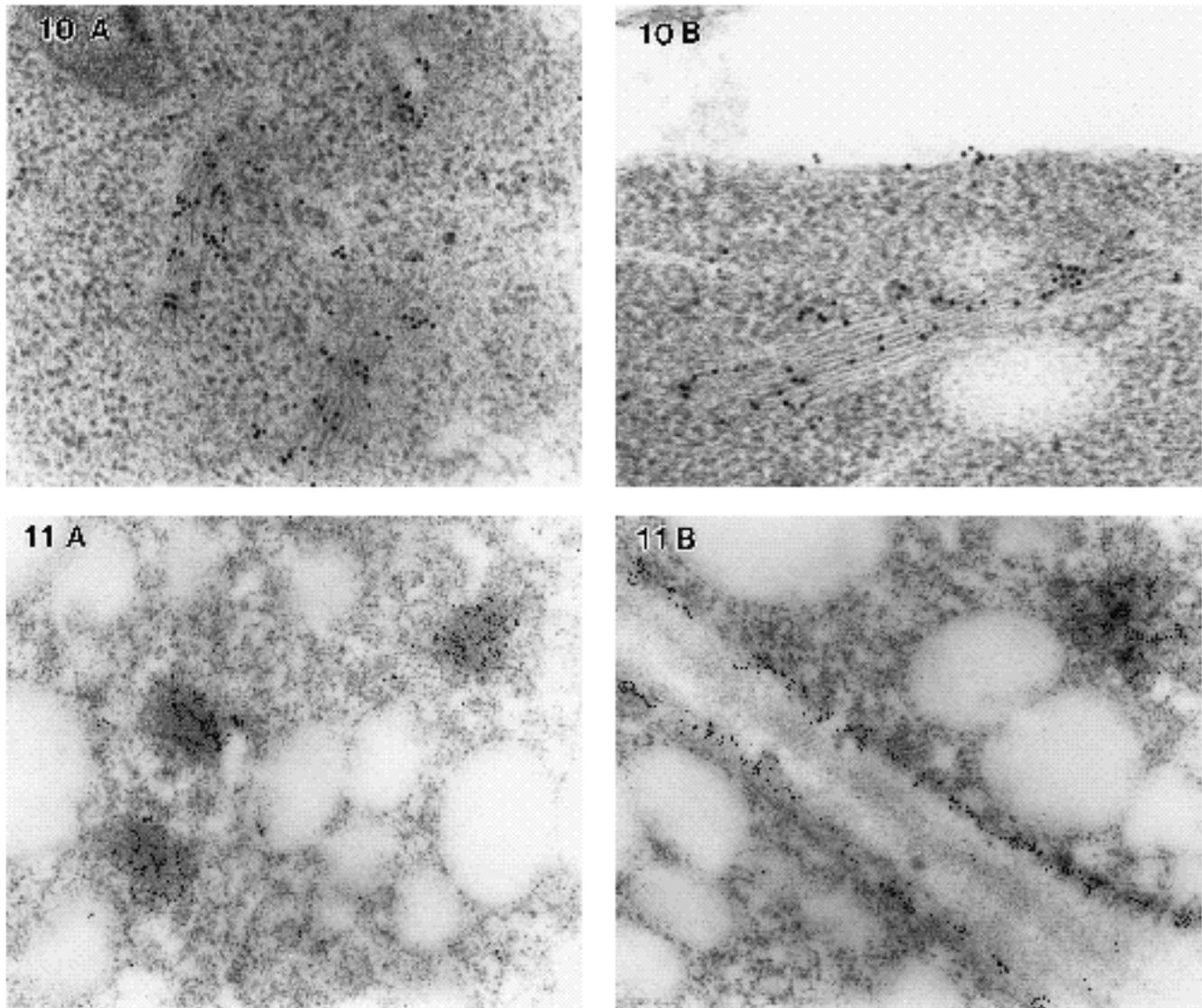
treated cells (Fig. 9B). Immunolabelling with JIM 84 preferentially stains these curved Golgi stacks. Vesicles near the Golgi were clear of gold particles (Fig. 7A).

However, in some of the 50  $\mu\text{g/ml}$  treated cells and in most of the 200  $\mu\text{g/ml}$  treated cells, Golgi stacks are rarely seen. Instead, delimited vesiculated areas of the cytoplasm occur as a common feature of BFA-induced modification (Figs 8, 9A,C). Labelling is always associated with these vesiculated areas and patches of gold are also seen at the periphery of these vesiculated areas (Figs 8B,C,D, 9A). This gold labels perturbed Golgi stacks which are localised at the periphery of these vesiculated areas (Figs 8C,D, 9A). Some gold particles are also observed inside the vesiculated areas, but labelling the cytosol not the vesicles. In other cases, where no Golgi stacks are observed, gold particles appear to be distributed randomly in the cytosol within

these vesiculated areas (Fig. 9C). These vesiculated areas of cytoplasm correspond to the circular fluorescent areas observed in the treated root cells by light microscopy (compare Figs 4A and 8A for maize root cells, or Figs 3A and 9C for onion root cells). The patchy staining at the plasma membrane generally remains after a 50  $\mu\text{g/ml}$  BFA treatment (Fig. 7B), but is variable after a 200  $\mu\text{g/ml}$  BFA treatment. Some cells exhibit a plasma membrane labelling similar to that observed in control cells (Fig. 7C), whilst others have a low level labelling (Fig. 8A,B). No labelling was observed in the endoplasmic reticulum of treated cells (Fig. 9D).

#### *Recovery following BFA treatment*

After a 50  $\mu\text{g/ml}$  BFA treatment, recovery from BFA was observed in both maize roots and onion roots (Fig. 11).



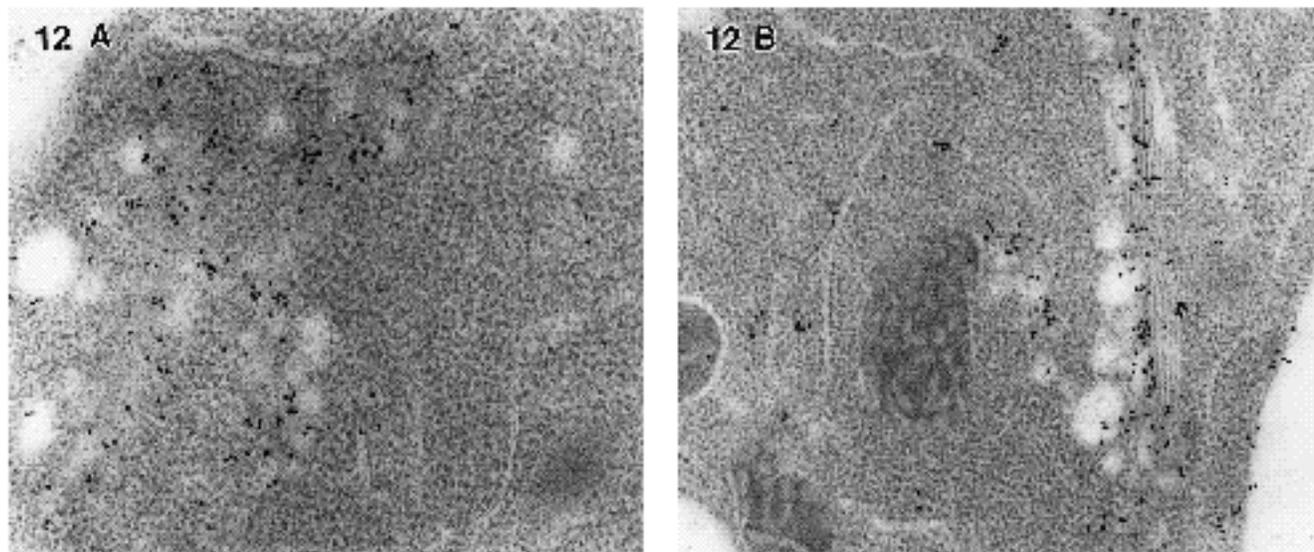
**Fig. 10.** Reappearance of immunogold labelling of the Golgi apparatus in maize cells following a recovery treatment after BFA. (A, B) Recovery following a 200  $\mu\text{g}/\text{ml}$  BFA treatment in maize root cells. Gold particles label the reformed Golgi stacks (A,  $\times 83\,500$ ; B,  $\times 108\,000$ ).

**Fig. 11.** Immunogold-labelling of the Golgi apparatus in onion roots following recovery after a 50  $\mu\text{g}/\text{ml}$  BFA treatment. (A) Golgi stacks are re-formed, and the JIM 84 epitope is preferentially localised on these stacks. The cytosol is clear of gold particles even around any remaining large vesicles ( $\times 42\,000$ ). (B) The plasma membrane exhibits heavier labelling compared to control cells after recovery from a 50  $\mu\text{g}/\text{ml}$  BFA treatment ( $\times 42\,000$ ).

Gold labelling is associated with small areas in the size range of Golgi stacks and often the stacks of Golgi cisternae are recognisable (Fig. 11A). In some cells, the reversibility coincided with a heavier staining of the plasma membrane (Fig. 11B). After a 200  $\mu\text{g}/\text{ml}$  BFA treatment and recovery, some cells continue to exhibit a localised partial vesiculation of the cytoplasm where gold particles were previously observed, although the vesiculated areas tend to disappear (Fig. 12A). Moreover, in the majority of cells, Golgi stacks were reformed (Figs 10A,B, 12B), often still surrounded by vesicles which are free of gold particles.

## Discussion

The observations performed on both onion and maize root cells with a wide range of BFA concentrations show that the dissociation of the Golgi apparatus induced by BFA is associated with a redistribution of a Golgi glycoprotein (the JIM 84 epitope) into discrete and vesiculated cytoplasmic compartments. Although slight variations in intensity from one cell to another and/or from one experiment to another occurred, the main events leading to such relocation of JIM 84 epitope can be summarized as in Fig. 13. The redistribution of the JIM 84 epitope in BFA-treated plant cells



**Fig. 12.** Recovery following a 200  $\mu\text{g/ml}$  BFA treatment of onion root cells. (A) In some cells gold particles are still localised in discrete areas in the cytoplasm. Note that the area is not as vesiculated as in BFA-treated cells, and the labelled area appears more structured ( $\times 64\ 000$ ). (B) Labelling is also associated with re-formed Golgi stacks. Note the labelling of the plasma membrane on the right hand side of the micrograph ( $\times 50\ 000$ ).

constitutes an original pattern when compared to the protein redistribution observed in animal cells.

#### *Brefeldin A studies in plant cells*

In animal cells, Brefeldin A has been shown to block protein secretion in pre-Golgi compartments and therefore has been widely used in the study of vesicle trafficking (Misumi et al., 1986; Fujiwara et al., 1988; Lippincott-Schwartz et al., 1989; Doms et al., 1989; see Pelham, 1991a and Klausner et al., 1992, for reviews). In plants, morphological observations of BFA-treated cells have revealed that BFA also affects the ultrastructure of the plant Golgi apparatus (Satiat-Jeuemaitre and Hawes, 1992), inducing a disassembly of the Golgi stacks. However, the fate of Golgi membrane components or secretory products usually sorted by the Golgi remained unknown. A biochemical study on protein secretion using BFA has been recently mentioned in the literature, suggesting that BFA has the same effects on plant protein secretion as the sodium/potassium ionophore monensin, in that it prevents the sorting of some proteins to the vacuoles, but unfortunately most of the data were not shown (Holwerda et al., 1992). The results presented here show for the first time that in plant cells BFA reversibly induces the redistribution of a Golgi glycoprotein from the Golgi stacks to other areas of the cytoplasm.

#### *The JIM 84 epitope as a Golgi marker*

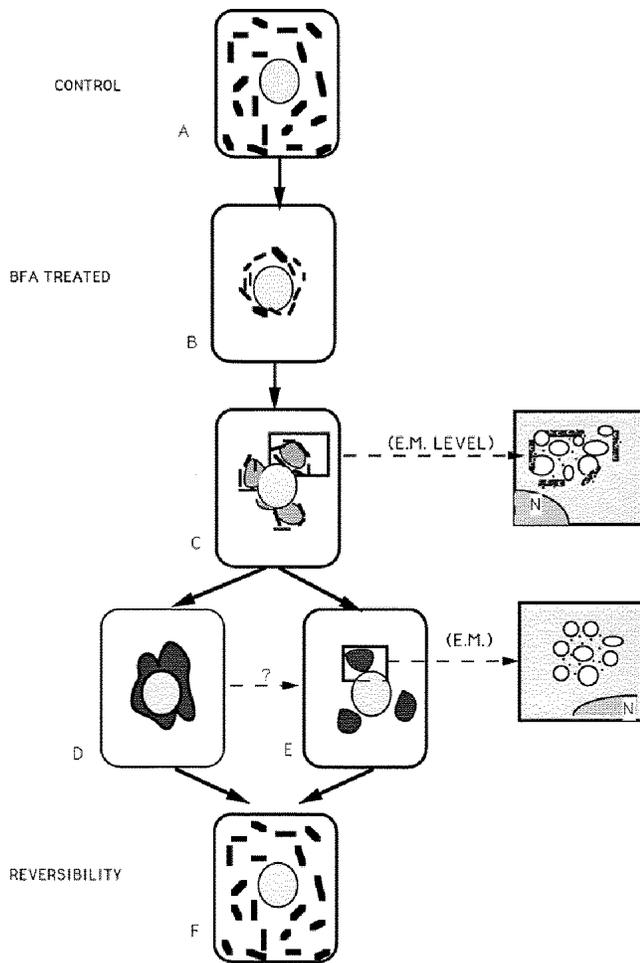
Recently a monoclonal antibody, JIM 84, has been shown to recognise the carbohydrate moiety of a glycoprotein associated with Golgi cisternae and plasma membranes in suspension culture cells (Horsley et al., 1993). The results presented here show that, in roots, the plasma membrane staining is slightly variable according to the root cells observed, probably reflecting variations in vesicular traffic. Due to this, in the present study we concentrated on the

JIM 84 epitope as a Golgi marker for studying the effects of BFA on Golgi-associated glycoproteins, and did not investigate the effect of BFA on secretion to the plasma membrane.

There are several factors which make the monoclonal antibody JIM 84 a good probe for studying the effects of drugs on the plant Golgi apparatus.

Firstly, from the observations we have performed, in the species studied to date, we can conclude that every Golgi stack is recognised by JIM 84. Thus, the JIM 84 epitope is likely to be ubiquitous in plants.

Secondly, JIM 84 gives a heavy labelling of the Golgi apparatus both by light and electron microscopy. At the electron microscope level, comparable pictures have only been published for the immunogold localisation of phytohemagglutinin (Greenwood and Chrispeels, 1985), where the association of the lectin with the cisternal stacks and Golgi vesicles was clearly demonstrated. In the other reports on Golgi immunostaining, labelling was often restricted to the swollen margins of the cisternae or to the budding vesicles, and not to the main body of the Golgi stack: for example the case of rhamnogalacturonan pectins (Moore and Staehelin, 1988), polygalacturonan pectins (Vian and Roland, 1991), xyloglucan hemicelluloses (Moore and Staehelin, 1988; Moore et al., 1991; Staehelin et al., 1991; Lainé et al., 1991), enzymes such as  $\alpha$ -amylase (Zingen-Sell et al., 1990), chitinase and  $\beta$ -(1-3)glucanase (see Hrazdina and Zobel, 1991 and references therein), lectins, and storage proteins such as vicilin (Craig and Goodchild, 1984b) or phaseolin and phytohemagglutinin (Greenwood and Chrispeels, 1985), and concanavalin A (Herman and Shannon, 1984). It is significant that none of these localisations were carried out at the light microscope level, a fact most likely attributable to the low level of signal from the probes.



**Fig. 13.** Diagram summarizing the dynamics of the subcellular redistribution of the JIM 84 epitope following BFA treatment. (A) In control cells labelled Golgi stacks are uniformly distributed in the cytoplasm. Following a BFA treatment, cortical Golgi stacks move to a perinuclear location (B), and they are often distorted. Following a longer exposure to the drug, or a higher concentration, the perturbed Golgi stacks circumscribe circular and vesiculated areas around the nucleus. The JIM 84 antibody still recognises these abnormal stacks, and also some components in the vesiculated areas (C). The ultimate response of the cells to a long or concentrated BFA treatment is the final disappearance of all forms of Golgi apparatus. The JIM 84 epitope remains trapped in the BFA-formed vesiculated areas usually around the nucleus (D, E). The process is reversible (F); after a recovery treatment, the BFA-induced vesiculated area tend to disappear, gold-labelled membranous structures and eventually fully recognisable Golgi comparable to the ones observed in control cells are seen. E.M., electron microscopy.

Finally, as JIM 84 immunoblots microsomal fractions from various plant tissues (Horsley et al., 1993), it is likely that it is associated with the Golgi membranes rather than being a soluble product. Therefore, it is to date a unique marker for the plant Golgi apparatus, as the previous studies on immunolabelling only concerned secretory products within the lumen of the cisternae.

#### *Brefeldin A induces redistribution of JIM 84 epitope to discrete cytoplasmic areas*

The degree of the BFA response appears to vary between roots and within cell populations of the same root. It could be that sensitivity to BFA varies between cell types or that there is a gradient of response from the cortex of the root to the central cells. Cryo-sectioning experiments are currently being undertaken to test these hypotheses.

From both immunofluorescence and electron microscopy we have shown that the effect of BFA on the plant Golgi apparatus is different from that reported for animal cells. Although in both cases the final result is the dissociation of the cisternal stacks, in animal cells the Golgi cisternae are reabsorbed back into the endoplasmic reticulum through microtubule-mediated tubulovesicular processes (Lippincott-Schwartz et al., 1989, 1990). In plant cells however, prior to dissociation, the Golgi stacks are apparently repositioned either in a perinuclear fashion or in circles of stacks in the cytosol. Disruption of the stacks appears to be a vesiculation process leaving discrete vesiculated areas in the cytosol. We have no evidence for tubular processes being formed from the Golgi cisternae. The morphological differences of BFA action may simply reflect the basic differences in the functioning of plant and animal Golgi (Griffing, 1991), the former, in most cases, being geared towards the secretion of polysaccharides, whilst in the latter the secretion of proteins is of greater quantitative significance and the Golgi apparatus tends to be more closely associated with the endoplasmic reticulum (Hawes et al., 1991).

The effects of BFA on the dynamics of the endomembranes can most readily be studied by immunofluorescence of Golgi marker proteins. Two pathways of redistribution of Golgi marker proteins have been described for animal BFA-treated cells. Firstly, initial reports suggested that both itinerant Golgi glycoproteins such as the vesicular stomatitis virus G protein (Doms et al., 1989) and resident Golgi proteins such as the *cis*/medial marker mannosidase II (Lippincott-Schwartz et al., 1989) are rapidly redistributed into the ER on BFA treatment. In the continued presence of BFA, these Golgi glycoproteins then cycle between the ER and an intermediate compartment (Lippincott-Schwartz et al., 1990). On the other hand, some cytosolic proteins associated with the Golgi apparatus have now been shown to be even more rapidly distributed on exposure to BFA, giving a diffuse staining pattern in the cytoplasm. This is the case for a 110 kDa peripheral cisternal membrane protein, that is dispersed into the cytosol prior to any effects on the redistribution of luminal proteins, or Golgi morphology (Donaldson et al., 1990). It is now apparent that the 110 kDa protein blocked by BFA is a coat protein ( $\beta$ -COP) of the Golgi non-clathrin-coated vesicles, involved in the membrane traffic through the Golgi complex (Duden et al., 1991a) and which has close homology to the clathrin-associated protein,  $\beta$ -adaptin (Duden et al., 1991b; Serafini et al., 1991; Waters et al., 1991). Supporting this evidence for the prime site of BFA action, it was shown that the drug blocks the assembly of non-clathrin-coated buds on the periphery of Golgi cisternae, thus preventing the shuttle of product down the cisternal stack and ultimately blocking secretion (Orci et al., 1991). Recently, a 200 kDa periph-

eral Golgi protein, probably involved in a non-clathrin-coating structure, has also been shown to redistribute into the cytosol as a result of BFA treatment (Narula et al., 1992).

Here we have shown that in plant cells, as a result of exposure to BFA, a cisternal membrane protein (the JIM 84 epitope) is not reabsorbed back in the endoplasmic reticulum as are some Golgi-resident proteins of animal cells, but is redistributed into the cytosol. However, unlike the 110 kDa or 200 kDa protein associated with animal cell Golgi, this epitope does not appear to be preferentially associated with cisternal vesicles and its redistribution is restricted to particular vesiculated areas instead being diffuse throughout the cytosol. From this investigation it is not clear whether these small vesicles are in fact derived from Golgi cisternae or result from some other effect of the BFA treatment.

Although the concentrations of BFA required to observe a dissociation of the plant Golgi apparatus are considerably higher than is the case with animal Golgi, the effect is also reversible, resulting in the re-formation and redistribution of discernable Golgi stacks and the incorporation of the JIM 84 epitope into the cisternae. However, it has been reported that a lower concentration (20 µg/ml) of BFA could prevent the transport of some Golgi proteins to the vacuoles (see caption of Fig. 4 of Holwerda et al., 1992). It is also interesting to note that on some occasions release from the effect of BFA results in a heavy labelling of the plasma membrane with JIM 84 (Fig. 11B). It is possible that the re-formation of the Golgi apparatus also coincides with an exocytotic event.

It is too early to comment on whether BFA has effects on other parts of the plant endomembrane system. In the literature on animal cells, recent reports suggest that BFA also affects the *trans*-Golgi network, endosomes and lysosomes (Lippincott-Schwartz et al., 1991; Wood et al., 1991; Reaves and Banting, 1992). It has also been reported that BFA does not perturb endocytosis, but inhibits transcytosis (Hunziker et al., 1991). Thus, it has been suggested that analysis of BFA action in animal cells may help reveal general principles that govern the ordered trafficking of vesicles between the components of the endomembrane system (Pelham, 1991a,b; Klausner et al., 1992). The present data indicate that BFA may also be a powerful tool in helping to elucidate some of the many unsolved problems regarding our understanding of the vectorial pathways of vesicle traffic in plants.

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## References

**Bednarek, S.Y. and Raikhel, N.V.** (1991). The barley lectin carboxyl-terminal propeptide is a vacuolar protein sorting determinant in plants. *Plant Cell* **3**, 1195-1206.

**Brummel, D.A., Camirand, A. and MacLachlan, G.A.** (1990). Differential distribution of xyloglucan glycosyl transferases in pea Golgi dictyosomes and secretory vesicles. *J. Cell Sci.* **96**, 705-710.

**Chrispeels, M.J.** (1991). Sorting of proteins in the secretory system. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42**, 21-53.

**Chrispeels, M.J. and Raikhel, N.** (1992). Short peptide domains target proteins to plant vacuoles. *Cell* **68**, 613-616.

**Craig, S. and Goodchild, D.J.** (1984a). A Golgi-mediated vicilin-accumulation in pea cotyledon cells is re-directed by monensin and nigericin. *Protoplasma* **122**, 91-97.

**Craig, S. and Goodchild, D.J.** (1984b). Periodate acid treatment of sections permits on-grid immunogold localization of pea seed vicilin in ER and Golgi. *Protoplasma* **122**, 35-44.

**Denecke, J., Botterman, J. and Deblaere, R.** (1990). Protein secretion in plant cells can occur via a default pathway. *Plant Cell* **2**, 51-59.

**Doms, R.W., Russ, G. and Yewdell, J.W.** (1989). Brefeldin A redistributes resident and itinerant Golgi proteins to the endoplasmic reticulum. *J. Cell Biol.* **109**, 61-72.

**Donaldson, J., Lippincott-Schwartz, J., Bloom, G., Kreis, T. and Klausner, R.D.** (1990). Dissociation of a 110-kD peripheral membrane protein from the Golgi apparatus is an early event in Brefeldin A action. *J. Cell Biol.* **111**, 2295-2306.

**Duden, R., Allan, V., and Kreis, T.** (1991a). Involvement of -COP in membrane traffic through the Golgi complex. *Trends Biochem. Sci.* **1**, 14-19.

**Duden, R., Griffiths, G., Frank, R., Argos, P. and Kreis T.** (1991b). -COP, a 110kD protein associated with non-clathrin-coated vesicles and the Golgi complex, shows homology to -adaptin. *Cell* **64**, 649-665.

**Fontes, E.B.P., Shank, B.B., Wrobel, R.L., Moose, S.P., O'Brian, G.R., Wurtzel, E.T. and Boston, R.S.** (1991). Characterization of an immunoglobulin binding protein in the maize *floury-2* endosperm mutant. *Plant Cell* **3**, 483-496.

**Fowke, L.C., Tanchak, M.A. and Galway, M.E.** (1991). Ultrastructure cytology of the endocytotic pathway in plants. In *Endocytosis, Exocytosis and Vesicle Traffic in Plants* (ed. C. Hawes, J. Coleman and D. Evans), pp. 15-41. Cambridge University Press, Cambridge, UK.

**Fujiwara, T., Oda, K., Yokota, S., Takatsuki A. and Ikehara Y.** (1988). Brefeldin A causes disassembly of the Golgi complex and accumulation of secretory proteins in the endoplasmic reticulum. *J. Biol. Chem.* **263**, 18545-18552.

**Greenwood, J.S. and Chrispeels, M.J.** (1985). Immunocytochemical localization of phaseolin and phytohemagglutinin in the endoplasmic reticulum and Golgi complex of developing bean cotyledons. *Planta* **165**, 295-302.

**Griffing, L.** (1991). Comparisons of Golgi structure and dynamics in plant and animal cells. *J. Elect. Microsc. Techn.* **17**, 179-199.

**Harris, N. and Watson M.** (1991). Vesicle transport to the vacuole and the central role of the golgi apparatus. In *Endocytosis, Exocytosis and Vesicle Traffic in Plants* (ed. C. Hawes, J. Coleman and D. Evans), pp. 143-164. Cambridge University Press, Cambridge, UK.

**Hawes, C., Evans, D. and Coleman, J.O.D.** (1991). An introduction to vesicle traffic in eukaryotic cells. In *Endocytosis, Exocytosis and Vesicle Traffic in Plants* (ed. C. Hawes, J. Coleman and D. Evans), pp. 1-15. Cambridge University Press, Cambridge, UK.

**Herman, E.M. and Shannon, L.M.** (1984). Immunocytochemical localization of concanavalin A in developing jack-bean cotyledons. *Planta* **161**, 97-104.

**Höfte, H., Faye, L., Dickinson, C., Herman, E.M. and Chrispeels, M.J.** (1991). The protein-body proteins phytohemagglutinin and tonoplast intrinsic protein are targeted to vacuoles in leaves of transgenic tobacco. *Planta* **184**, 431-437.

**Holwerda, B. C., Padgett, H. and Rogers, J. C.** (1992). Proaleurain vacuolar targeting is mediated by short contiguous peptide interactions. *The Plant Cell* **4**, 307-318.

**Horsley, D., Coleman, J., Evans, D., Crooks, K., Peart, J., Satiat-Jeunemaitre, B. and Hawes, C.** (1993). A monoclonal antibody, JIM 84, recognises the Golgi apparatus and plasma membrane in plant cells. *J. Exp. Bot.* **44**, in press.

**Hrazdina, G. and Zobel, A.M.** (1991). Cytochemical localization of enzymes in plant cells. *Int. Rev. Cytol.* **129**, 269-322.

**Hunziker, W., Whitney, J.A. and Mellman, I.** (1991). Selective inhibition of transcytosis by Brefeldin A in MDCK cells. *Cell* **67**, 617-627.

**Klausner, R.D., Donaldson, J.G. and Lippincott-Schwartz, J.** (1992). Brefeldin A: insights into the control of membrane traffic and organelle structure. *J. Cell Biol.* **116**, 1071-1092.

**Lainé, A.C., Gomord, V. and Faye L.** (1991). Xylose-specific antibodies

- as markers of subcompartmentation of terminal glycosylation in the Golgi apparatus of sycamore cells. *FEBS Lett.* **295**, 179-184.
- Lippincott-Schwartz, J., Yuan, L., Bonifacino, J. and Klausner, R.** (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.
- Lippincott-Schwartz, J., Donaldson, J., Schweizer, A., Berger, E., Hauri, H., Yuan, L. and Klausner, R.** (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.
- Lippincott-Schwartz, J., Yuan, L., Tipper, C., Amherd, T.M., Orci, L. and Klausner, R.** (1991). Brefeldin A's effects on endosomes, lysosomes, and the TGN suggest a general mechanism for regulating organelle structure and membrane traffic. *Cell* **67**, 601-616.
- Matsuoka, K. and Nakamura, K.** (1991). Propeptide of a precursor to a plant vacuolar protein required for vacuolar targeting. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 834-838.
- Misumi, Y., Misumi, Y., Miki, K., Takatsuki, G. and Ikehara, Y.** (1986). Novel blockade by Brefeldin A of intracellular transport of secretory proteins in cultured rat hepatocytes. *J. Biol. Chem.* **261**, 11398-11403.
- Moore, P. J., and Staehelin, L.A.** (1988). Immunogold localization of the cell-wall-matrix polysaccharides rhamnogalacturonan I and xyloglucan during cell expansion and cytokinesis in *Trifolium pratense* L.; implication for secretory pathways. *Planta* **174**, 433-445.
- Moore, P.J., Swords, K.M., Lynch, M.A. and Staehelin, L.A.** (1991). Spatial organization of the assembly pathways of glycoproteins and complex polysaccharides in the Golgi apparatus of plants. *J. Cell Biol.* **112**, 589-602.
- Napier, R.M., Fowke, L.C., Hawes, C., Lewis, M. and Pelham, H.R.B.** (1992). Immunological evidence that plants use both HDEL and KDEL for targeting proteins to the endoplasmic reticulum. *J. Cell Sci.* **102**, 261-271.
- Narula, N., McMorro, I., Plopper, G., Doherty, J., Matlin, K., Burke, B. and Stow, J.** (1992). Identification of a 200-kD, Brefeldin-sensitive protein on Golgi membranes. *J. Cell Biol.* **117**, 27-38.
- Neuhaus, J.M., Sticher, L., Meins, F. Jr and Boller, T.** (1991). A short C-terminal sequence is necessary and sufficient for the targeting of chitinases to the plant vacuole. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10362-10366.
- Orci, L., Tagaya, M., Amherd, T.M., Perrelet, A., Donaldson, J., Lippincott-Schwartz, J., Klausner, R. and Rothman, J.** (1991). Brefeldin A, a drug that blocks secretion, prevents the assembly of non-clathrin-coated buds on Golgi cisternae. *Cell* **64**, 1183-1195.
- Pelham, H.R.** (1991a). Multiple targets for Brefeldin A. *Cell* **67**, 449-451.
- Pelham, H.R.** (1991b). Recycling of proteins between the endoplasmic reticulum and Golgi complex. *Curr. Opin. Cell Biol.* **3**, 585-591.
- Reaves, B. and Banting, G.** (1992). Perturbation of the morphology of the trans-Golgi network following Brefeldin A treatment: redistribution of a TGN-specific integral membrane protein, TGN38. *J. Cell Biol.* **116**, 85-94.
- Saalbach, G., Jung, R., Kunze, G., Saalbach, I., Adler, K. and Müntz, K.** (1991). Different legumin protein domains act as vacuolar targeting signals. *The Plant Cell* **3**, 695-708.
- Satiat-Jeunemaitre, B. and Hawes, C.** (1992). Reversible dissociation of the plant Golgi apparatus by Brefeldin A. *Biol. Cell* **74**, 325-328.
- Serafini, T., Stenbeck, G., Brecht, A., Lottspeich, F., Orci, L., Rothman, J.E. and Wieland, F.T.** (1991). A coat subunit of Golgi-derived, non-clathrin-coated vesicles with homology to the clathrin-coated vesicle coat protein  $\beta$ -adaptin. *Nature* **349**, 215-220.
- Staehelin, L.A., Giddings, T.H., Levy, S., Lynch, M.A., Moore, P.J. and Swords, K.M.M.** (1991). Organisation of the secretory pathway of cell wall glycoproteins and complex polysaccharides in plant cells. In *Endocytosis, Exocytosis and Vesicle Traffic in Plants* (ed. C. Hawes, J. Coleman, and D. Evans), pp. 183-198. Cambridge University Press, Cambridge, UK.
- VandenBosch, K.A.** (1991). Immunogold labelling. In *Electron Microscopy of Plant Cells* (ed J. Hall and C. Hawes.), chapter 5. Academic Press.
- Vian, B. and Roland, J.C.** (1991). Affinodetection of the sites of formation and further distribution of polygalacturonans and native cellulose in growing plant cells. *Biol. Cell* **71**, 43-55.
- 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.
- Wood, S.A., Park, J.E. and Brown, W.J.** (1991). Brefeldin A causes a microtubule-mediated fusion of the trans-Golgi network and early endosomes. *Cell* **67**, 591-600.
- Zingen-Sell, I., Hillmer, S., Robinson, D.G. and Jones, R.L.** (1990). Localization of  $\alpha$ -amylase isozymes within the endomembrane system of barley aleurone. *Protoplasma* **154**, 16-24.

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