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Transport of horseradish peroxidase from the cell surface to the Golgi in insulin-secreting cells: preferential labelling of cisternae located in an intermediate position in the stack

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We have used serial sectioning to study the topology of Golgi cisternae in insulin-secreting cells during secretion-stimulated endocytotic uptake of exogeneous horseradish peroxidase (HRP). HRP-labelled cisternae were followed on several series of consecutive sections. This revealed that labelled cisternae could always be traced to a position in the Golgi stack intermediate between the *cis* and the *trans* poles. This occurred in spite of the apparent *cis* or *trans* locations of HRP-containing cisternae on some sections. The latter images could be explained by the lack of the true *cis* or *trans* (clathrin-coated) cisternae at certain levels of the stack.

Key words: endocytosis/Golgi apparatus/horseradish peroxidase/Golgi cisternae

Introduction

Exocytotic release of secretory granules from the insulin-secreting B-cell is a trigger for plasma membrane retrieval by endocytosis (Orci *et al.*, 1973). A similar process has been demonstrated in numerous cell types where exocytosis takes place (for review see Farquhar and Palade, 1981). In order to follow the fate of retrieved membrane vesicles (and their content), electron-dense markers of endocytosis such as dextran, horseradish peroxidase (HRP), anionic or cationic ferritin were used. These studies revealed a number of pathways for endocytotic membrane traffic (for review see Farquhar and Palade, 1981; Farquhar, 1985). One such pathway in secretory cells involves the Golgi apparatus (Pelletier, 1973; Mata and David-Ferreira, 1973; Orci in Meldolesi, 1974; Herzog and Farquhar, 1977; Orci *et al.*, 1978). The exact site of relocation of the marker at this level is, however, unsettled. In some instances, the endocytosed marker has been found throughout most cisternae of the Golgi stack, but it is often described as labelling preferentially the *trans* side of the stack. These seemingly diverging results may be due in part to the difficulty of interpreting the complex topology of the stack and associated vesicles when observed on individual thin sections. We have re-examined this issue in insulin-secreting cells labelled with HRP and have used serial sections to follow the location and extension of the Golgi cisternae containing the endocytosed marker. We have found that a single HRP-labelled cisterna may assume different apparent locations with respect to the *cis*–*trans* polarity, but that all HRP-labelled cisternae can ultimately be traced to an intermediate position in the stack.

Results

In glucose-stimulated B cells examined after a 15-min incubation with HRP, labelled vesicles were found throughout the entire cytoplasm, but with a preferential location in the Golgi area. In addition to cytoplasmic vesicles, numerous secondary lysosomes were also labelled by the reaction product. At the level of the Golgi apparatus, some cisternae contained HRP whereas others did not (Figure 1). The distribution of the labelled cisternae within the Golgi stack varied on individual sections (Figure 1), but became evident when Golgi stacks were examined by serial sectioning. Figure 2 shows six successive serial sections through such a Golgi stack: a single HRP-labelled cisterna that appeared to delimit the *trans* pole on one section could clearly be traced to an intermediate position between the *cis* and *trans* poles when identified on consecutive sections. Fifteen complete Golgi stacks were examined in this way and the peroxidase-labelled cisternae could always be traced to an intermediate location; this was also true for individual HRP-labelled cisternae which were seen on some sections to apparently delimit the *cis* pole (Figure 3). Similar observations on the apparent *cis* or *trans* location of HRP-labelled cisternae could be made as early as 5 min following i.v. injection of HRP *in vivo* (data not shown). The change from an apparent *trans*- most location to an intermediate one could also be evidenced with thiamine pyrophosphatase as a marker. On some sections, a TPPase-positive cisterna appeared to delimit the *trans* pole; this pole was occupied by a TPPase-negative cisterna on successive sections, while the marked compartment was seen between the *cis* and *trans* poles (Figure 4). On serial sections, a characteristic and reliable *trans* Golgi marker was the presence

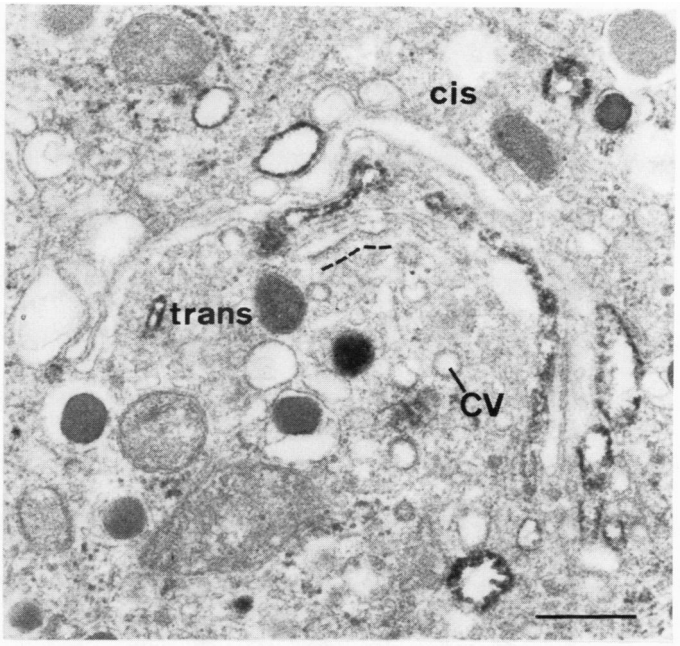

Fig. 1. Thin section of the Golgi area of an insulin-secreting cell during endocytotic uptake of HRP. The curved Golgi stack contains several HRP-labelled cisternae with different locations with respect to *cis*–*trans* polarity. A coated cisterna without HRP labelling is indicated by the dotted line at the *trans*- most pole. CV, coated vesicle. The bar represents 0.5 μm. × 26 000.

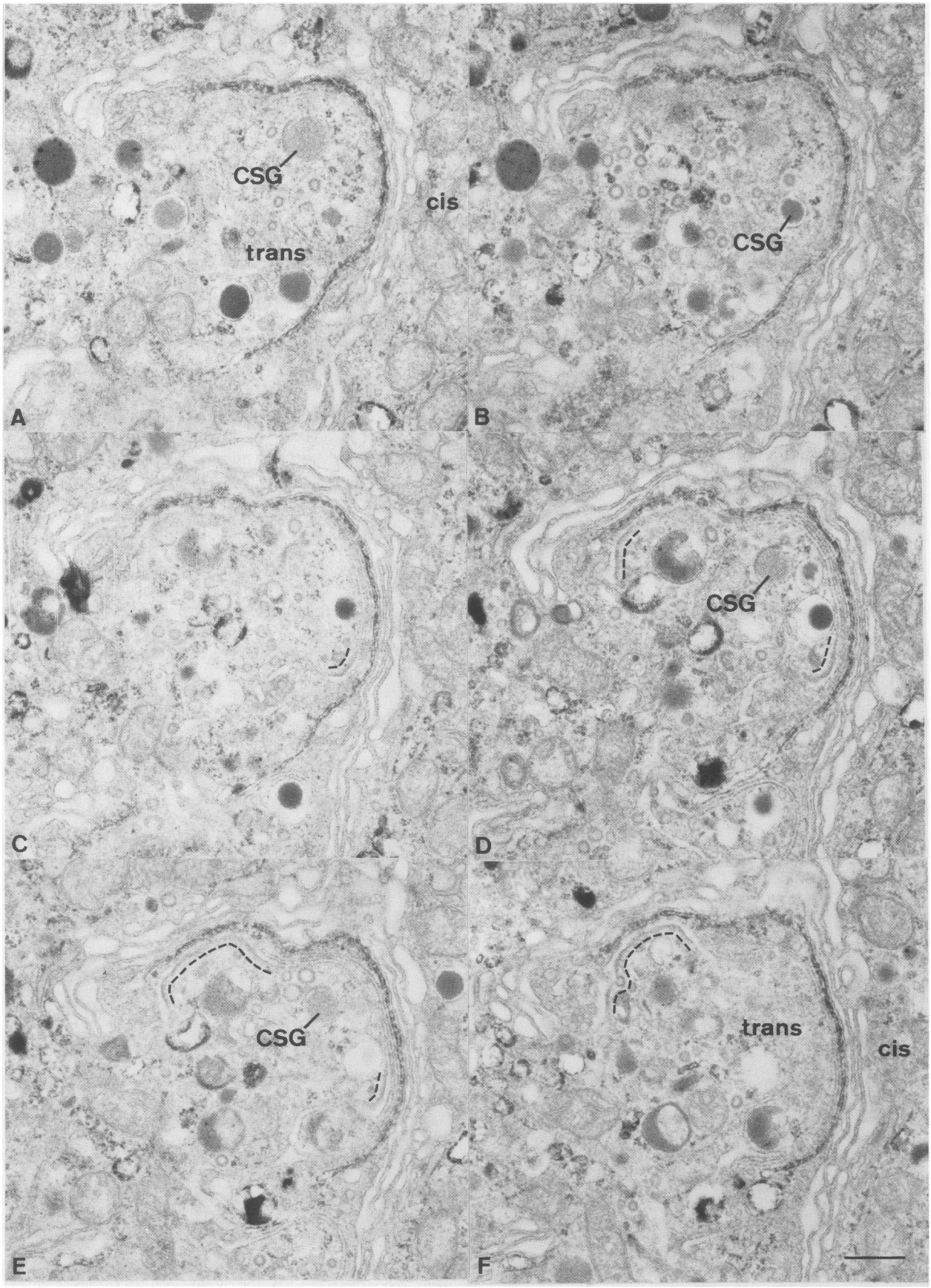

Fig. 2. Series of six consecutive serial sections (of a total of 14) showing the different apparent locations of a single HRP-labelled cisterna with respect to the overall *cis*–*trans* polarity. The *trans* pole is characterized by segments of coated cisternae (dotted lines) and/or by the presence of coated secretory granules (CSG). The cisterna which appears to delimit the *trans* pole on sections **A** and **B** occupies an intermediate position between the *cis* and *trans* poles on successive sections **C**–**F**. The bar represents 0.5 μm. × 22 000.

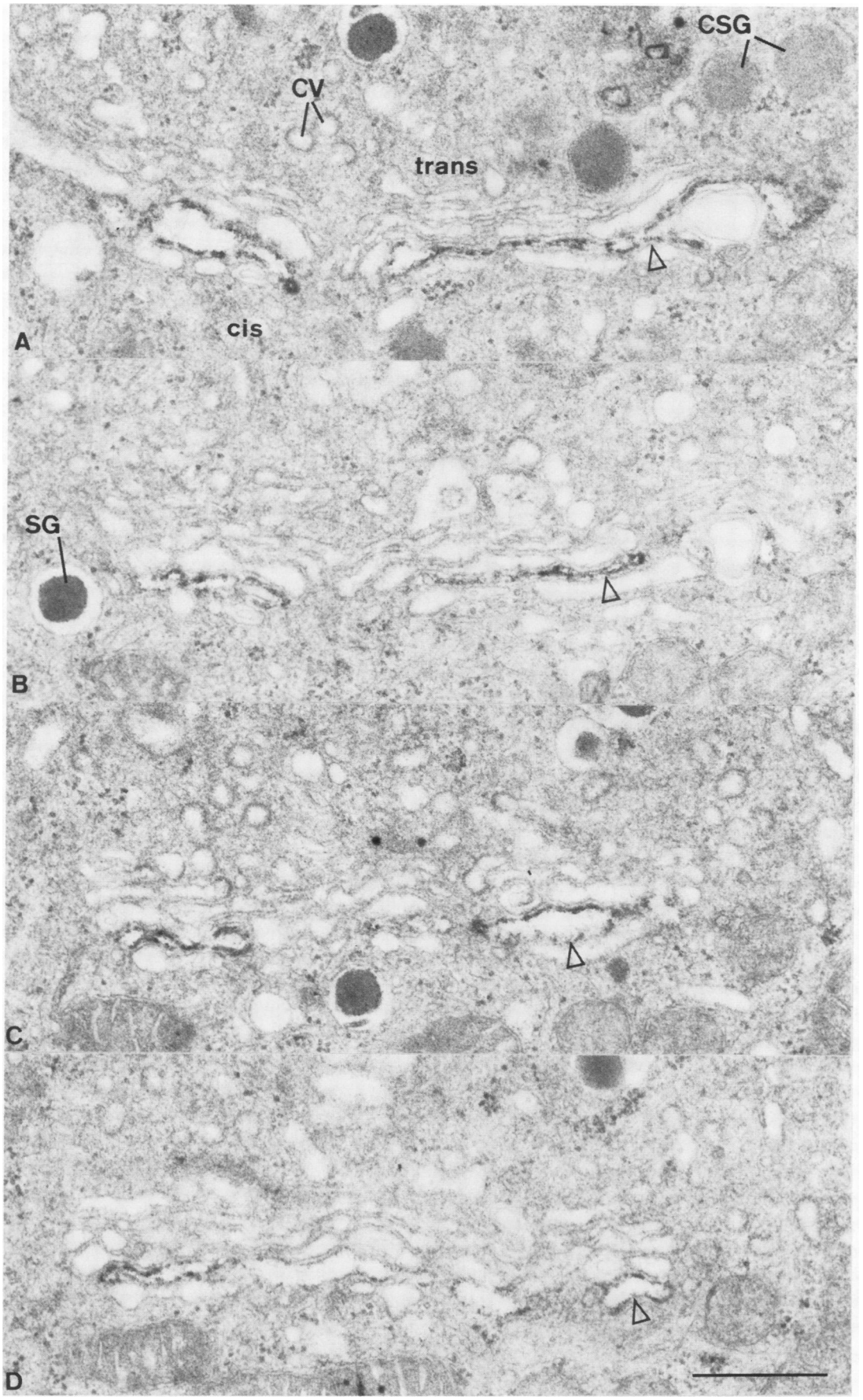

Fig. 3. Sections No. 4, 6, 8 and 10 (of a total of 10) from a HRP-labelled Golgi stack. A labelled cisterna (white arrowhead) in an intermediate position between the *cis* and *trans* poles on sections **A**–**C** appears to delimit the *cis* pole on section **D**. CSG, coated secretory granules; SG, non-coated granule; CV, coated vesicles. The bar represents 0.5 μm. × 46 000.

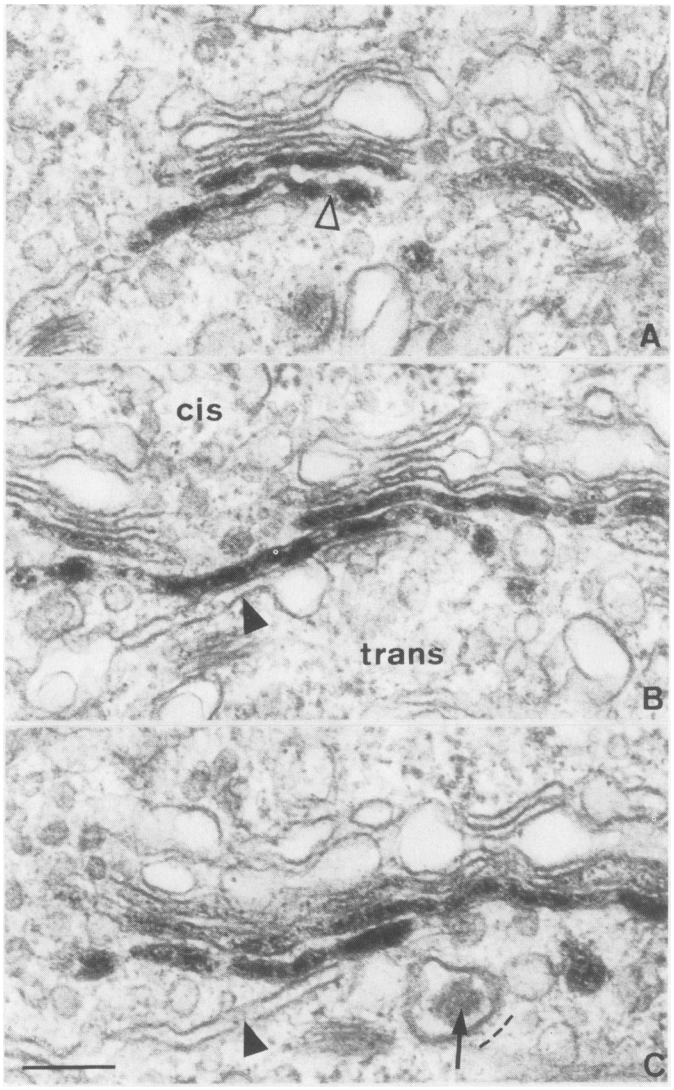

Fig. 4. Three consecutive sections (of a total of 11) from a thiamine-pyrophosphatase (TPPase)-stained B cell. A cisterna containing the black reaction product of TPPase appears to delimit part of the *trans* pole of the Golgi stack on section (**A**) (white arrowhead). TPPase-free cisternae become evident in a *trans*- most position on successive sections (black arrowhead) (**B,C**) and TPPase-containing cisternae are now seen between the *cis* and *trans* poles. The arrow indicates a maturing secretory granule with a coat on part of its limiting membrane (dotted line). The bar represents 0.2 μm. × 62 000.

of clathrin-coated cisternae (Figures 1 and 2) (see also Figure 13 in Orci, 1982; Figure 2 in Orci *et al.*, 1985). These cisternae, at the level of which condensation of secretory material occurs, were invariably in the *trans*- most position and never contained HRP. This observation is in keeping with recent data showing that newly formed secretory granules freshly released from the *trans* Golgi pole are not labelled with HRP (Sawano *et al.*, 1986). These data also argue against the shift of entire HRP-labelled cisternae from intermediate to *trans* position during prolonged HRP incubation.

Discussion

Following the application of electron-dense markers to trace the fate of endocytotic vesicles in a variety of secretory cells, a number of different pathways for intracellular traffic have been described (for review see Farquhar and Palade, 1981; Farquhar, 1985). In the various cell types examined, the Golgi apparatus was consistently described to be a site of marker's uptake, but the precise topographical distribution of Golgi labelling has remained unclear. The Golgi stack is subdivided into *cis* and *trans* compartments on the basis of topographical and cytochemical evidence (for review, see Farquhar, 1985) and more recently, a functionally distinct *medial* region has been detected in studies on transport of viral membrane protein (Griffiths *et al.*, 1983).

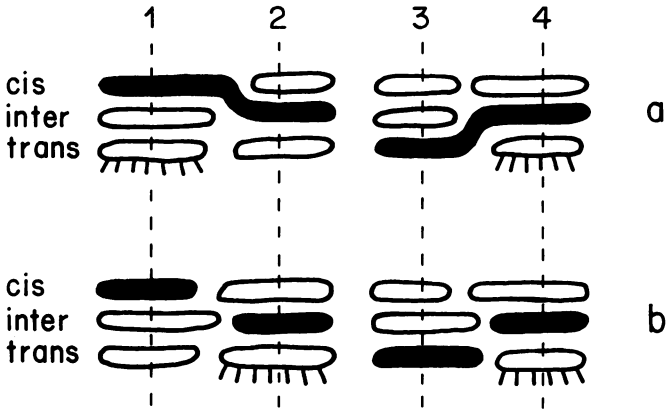

Fig. 5. Schematic drawing of the possible distribution and shape of individual cisternae in a Golgi stack to account for the different apparent locations of HRP labelling (black). The number of cisternae in the stack was set arbitrarily to a minimum of three. Dotted lines **1–4** represent different planes of sectioning across the width of the stack. *Trans*- most cisternae are characterized by clathrin-coated segments.

In the present report, we show that during endocytosis of HRP by insulin-secreting cells, HRP can be traced to Golgi cisternae located between the *cis* and *trans* poles. This contrasts with previous data indicating predominant HRP or dextran labelling of *trans* Golgi cisternae (for review see Farquhar and Palade, 1981; Farquhar, 1985). To account for our findings, i.e. that an intermediate cisterna can apparently delimit a *trans* or a *cis* Golgi pole, one has to envisage the existence of complex cisternae (or a network of communicating cisternae) extending across the stack (Figure 5, drawing a), and/or a different relative position of the cisternae along the width of the stack (Figure 5, drawing b). By this way, one could explain why a cisterna belonging to the intermediate region of the stack (Figure 5, sections 2 and 4) can be seen to apparently delimit a *cis* or *trans* pole on consecutive sections (Figure 5, sections 1 and 3). In the latter cases therefore, *cis* and *trans* connotations are misleading since they refer to an incomplete stack. In section 3 for example, the stack is lacking the *trans* marker represented by the clathrin coat (Orci *et al.*, 1985). These observations emphasize the need for 3-D analysis in order to define the topology of Golgi components.

Whether HRP-labelled elements represent resident Golgi cisternae or an endosomal compartment with a specific distribution in the Golgi stack is not known. In functional terms, relocation of endocytosed membrane (and content) to an intermediate rather than a *trans* Golgi compartment would be consistent with the concept that definitive sorting of products from the Golgi stack is a *trans* event (Griffiths *et al.*, 1985; Kelly, 1985). *Trans* cisternae are characteristically clathrin-coated (Orci *et al.*, 1985). The recycling of endosomal constituents directly at the *trans* level would require additional recognition mechanisms to separate incoming (recycled) constituents from those being sorted to various cellular destinations; such complexity would be rendered unnecessary if recycling involves a presorting, intermediate Golgi compartment.

Materials and methods

Islets of Langerhans were isolated from the pancreas of SIVZ Wistar rats by collagenase digestion (Lacy and Kostianovsky, 1967). The freshly isolated islets were equilibrated in Krebs–Ringer bicarbonate buffer containing 5 mg/ml BSA and a stimulatory concentration (16.7 mM) of glucose for 30 min. After equilibration, the islets were incubated for 15 min in the same medium containing 10 mg/ml HRP (Sigma, Type II). All incubations were carried out at 37°C in an atmosphere of 95% O_2 and 5% CO_2. After HRP exposure, islets were fixed for 1 h with 2% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4.

Following fixation, isolated islets were rinsed overnight in buffer and cut into 40-μm slices with a tissue chopper (Sorvall Inc., Newton, CT). HRP cytochemistry was performed according to Graham and Karnovsky (1966). Slices were post-fixed for 1 h in 1% phosphate-buffered osmium tetroxide, dehydrated in ethanol and embedded in Epon. Serial thin sections were cut on an LKB Ultratome with a diamond knife, and collected on one (1 × 2 mm) hole grids covered with a film of carbon-coated Parlodion.

Thiamine pyrophosphatase cytochemistry was carried out according to Novikoff and Goldfischer (1961) on pellets of isolated islets.

Acknowledgements

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References

Farquhar,M.G. (1985) *Annu. Rev. Cell Biol.*, **1**, 447–488.
Farquhar,M.G. and Palade,G.E. (1981) *J. Cell Biol.*, **91**, 77s–103s.
Graham,R.C.,Jr and Karnovsky,M.J. (1966) *J. Histochem. Cytochem.*, **14**, 291–302.
Griffiths,G., Quinn,P. and Warren,G. (1983) *J. Cell Biol.*, **96**, 835–850.
Griffiths,G., Pfeiffer,S., Simons,K. and Matlin,K. (1985) *J. Cell Biol.*, **101**, 949–964.
Herzog,V. and Farquhar,M.G. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5073–5077.
Kelly,R.B. (1985) *Science*, **230**, 25–32.
Lacy,P.E. and Kostianovsky,M. (1967) *Diabetes*, **16**, 35–39.
Mata,L.R. and David-Ferreira,J.F. (1973) *J. Microsc. (Paris)*, **17**, 103–106.
Meldolesi,J. (1974) In Ceccarelli,B., Clementi,F. and Meldolesi,J. (eds), *Advances in Cytopharmacology*. Raven Press, NY, Vol. **2**, (p. 83, Figure 6, by L. Orci).
Novikoff,A.B. and Goldfischer,S. (1961) *Proc. Natl. Acad. Sci. USA*, **47**, 803–810.
Orci,L. (1982) *Diabetes*, **31**, 538–565.
Orci,L., Perrelet,A. and Gorden,P. (1978) *Recent Prog. Horm. Res.*, **34**, 95–121.
Orci,L., Malaisse-Lagae,F., Ravazzola,M., Amherdt,M. and Renold,A.E. (1973) *Science*, **181**, 561–562.
Orci,L., Ravazzola,M., Amherdt,M., Louvard,D. and Perrelet,A. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 5385–5389.
Pelletier,G. (1973) *J. Ultrastr. Res.*, **43**, 445–459.
Sawano,F., Ravazzola,M., Amherdt,M., Perrelet,A. and Orci,L. (1986) *Exp. Cell Res.*, **164**, 174–182.

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