GLUT2 Surface Expression and Intracellular Transport via the Constitutive Pathway in Pancreatic β Cells and Insulinoma: Evidence for a Block in Trans-Golgi Network Exit by Brefeldin A

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Abstract. The biosynthesis, intracellular transport, and surface expression of the β cell glucose transporter GLUT2 was investigated in isolated islets and insulinoma cells. Using a trypsin sensitivity assay to measure cell surface expression, we determined that: (a) greater than 95% of GLUT2 was expressed on the plasma membrane; (b) GLUT2 did not recycle in intracellular vesicles; and (c) after trypsin treatment, reexpression of the intact transporter occurred with a t1/2 of ~7 h.

Kinetics of intracellular transport of GLUT2 was investigated in pulse-labeling experiments combined with glycosidase treatment and the trypsin sensitivity assay. We determined that transport from the endoplasmic reticulum to the trans-Golgi network (TGN) occurred with a t1/2 of 15 min and that transport from the TGN to the plasma membrane required a similar half-time. When added at the start of a pulse-labeling experiment, brefeldin A prevented exit of GLUT2 from the endoplasmic reticulum. When the transporter was first accumulated in the TGN during a 15-min period of chase, but not following a low temperature (22°C) incubation, addition of brefeldin A (BFA) prevented subsequent surface expression of the transporter. This indicated that brefeldin A prevented GLUT2 exit from the TGN by acting at a site proximal to the 22°C block. Together, these data demonstrate that GLUT2 surface expression in β cells is via the constitutive pathway, that transport can be blocked by BFA at two distinct steps and that once on the surface, GLUT2 does not recycle in intracellular vesicles.

GLUT2 is a facilitated diffusion glucose transporter characterized by a restricted tissue distribution: it is present in the sinusoidal membrane of hepatocytes, the basolateral membrane of intestine and kidney epithelial cells, and in pancreatic β cells (Thorens, 1992). Functionally, GLUT2 differs from the other facilitated diffusion glucose transporter isoforms by a relatively high Michaelis constant for glucose (Km = ~17 mM) (Johnson et al., 1990a). The expression of GLUT2 in pancreatic β cells has been suggested to be important for the normal glucose sensitivity of these cells (Unger, 1991; Thorens, 1992). Importantly, the expression of this transporter is reduced or suppressed in glucose-unresponsive β cells from diabetic rats and mice, a phenomenon that may participate in the β cell dysfunctions associated with diabetes (Johnson et al., 1990b; Orci et al., 1990; Thorens et al., 1990, 1992). Decreased expression of GLUT2 in diabetes is observed only in β cells while there is no change or rather an elevation of its expression in the liver, intestine, and kidney. While in most situations decreased expression of GLUT2 correlates with a decrease in its mRNA levels, decreased GLUT2 expression in dexamethasone-treated rats has been reported to be controlled at the translational or posttranslational level (Ogawa et al., 1992). Despite its important role in β cell physiology and its regulated expression in diabetes, a detailed knowledge of GLUT2 biosynthesis, intracellular transport, and surface expression is still lacking.

Pancreatic β cells have both a constitutive and regulated pathway (Kelly, 1985) for protein surface expression or secretion. Insulin biosynthesis, targeting to the secretory pathway, and regulated secretion by exocytosis have been extensively studied (Steiner, 1977). In particular, exit of proinsulin from the TGN towards the regulated pathway and its parallel conversion into insulin has been well described (Orci et al., 1985, 1987). Comparatively little is known, however, about the biosynthesis of endogenous β cell proteins following the constitutive pathway. Infection of islet cell monolayers with influenza or vesicular stomatitis viruses resulted in polarized cell surface budding of viral particles.
This indicated that the constitutive pathway can deliver membrane proteins in a polarized way (Lombardi et al., 1985). Localization of GLUT2 to microvilli present on lateral membranes of β cells was another indication of the polarity of the β cell surface (Orci et al., 1989).

Here we studied GLUT2 surface expression in pancreatic β cells using a protease sensitivity assay, and we investigated its intracellular transport by biosynthetic labeling experiments using the INS-1 insulinoma cell line. We demonstrated that GLUT2 is permanently expressed at the cell surface and does not recycle in intracellular vesicles. We followed GLUT2 intracellular transport to the cell surface via the constitutive pathway, and we showed that brefeldin A (BFA) blocked this transport at two different steps: at the exit of the endoplasmic reticulum, as already extensively described for a number of proteins, and also at the exit from the TGN before reaching the plasma membrane.

Materials and Methods

Materials

Tissue culture medium and supplements were from Gibco/BRL (Gaithersburg, MD). Collagenase CLS4 was from Worthington Biochem Corp. (Freehold, NJ). Ficoll 400 d from Sigma Immunochemicals (St. Louis, MO). [35S]Methionine from Amersham Corp. (Arlington Heights, IL). Neuraminidase from vibrio cholerae from Boehringer Corp. Mannheim (Indianapolis, IN) and endo-β-glycosaminidase-H from Boehringer Mannheim Corp. or New England Biolabs (Beverly, MA). BFA was a gift from Drs. Römer and Rissi, Sandoz Preclinical Research, Basel, Switzerland.

Islet Isolation and Cell Culture

Pancreatic islets were isolated from Sprague-Dawley rats weighing 190-230 g by collagenase digestion of the pancreas followed by separation on discontinuous Ficoll gradients, according to the method of Gotth et al. (1987). Before the experiments, islets were kept at a concentration of 5-10 islets per ml in culture medium (RPMI1640, 10 mM Hepes, pH 7.4, 2 mM glutamine, 1 mM pyruvate, 50 μM β-mercaptoethanol, and 10% FCS for at least 18 h to allow them to recover from the isolation procedure. The insulinoma cell line INS-1 (Asfari et al., 1992) (passages 100-120) was kept in the same culture medium. Incubations were run at 37°C and in a humidified atmosphere containing 5% CO2.

Antibodies, Western Blot Analysis and Trypsin Digestion

Antipeptide antibodies to GLUT2 have been described previously (Thorens et al., 1988). Briefly, the carboxy-terminal antibody was raised against a peptide corresponding to amino acids 513-522 of rat GLUT2 and the antibody to the first exoplasmic loop of rat GLUT2 was against a peptide corresponding to amino acids 47-60. For Western blot analysis of GLUT2, islets or INS-1 cells were lysed in a buffer containing 80 mM Tris-HCl, pH 6.8, 5% SDS, 5 mM EDTA, 0.2 mM N-ethyl maleimide (NEM), 1 mM PMSF. The lysates were sonicated for 30 s in the cup of a sonicator (Branson Ultrasonics Corp., Danbury, CT), separated by electrophoresis on SDS-containing 7.5% polyacrylamide gels, transferred to nitrocellulose filters. Detection of GLUT2 was performed as previously described (Thorens et al., 1988) except that we used the enhanced chemiluminescence detection system (ECL) from Amersham Corp. with the primary antibodies diluted 1:2,000. For trypsin digestion, islets or INS-1 cells were washed three times in HBSS containing 20 mM Hepes, pH 7.4. Incubations were in the same buffer with 20 μg/ml of TPCK-treated trypsin for 30 min at 4°C except when otherwise stated. Islets were incubated by groups of 20-50 in 500 μl of the above buffer and INS-1 cells in 60-mm tissue culture dishes in 2 ml of the same solution. The digestions were stopped by three washes in the presence of 1 mM PMSF before preparation of cell lysates for Western blot analysis or GLUT2 immunoprecipitation.

Biosynthetic Labeling and Immunoprecipitation

For biosynthetic labeling, INS-1 cells were grown in 60-mm tissue culture dishes, washed three times in HBSS, and incubated in 1 ml of RPMI 1640 culture medium lacking methionine, plus the complement described above and 10% dialyzed calf serum. Cells were preincubated for 30 min at 37°C, before addition of [35S]methionine at a concentration of 100 μCi/ml. In all experiments, the pulse labeling was for 5 min at 37°C. The cells were then quickly transferred to ice, the medium removed, and the cells washed with ice-cold HBSS. The cells were then either kept on ice before further processing or they received prewarmed medium containing 2 mM cold methionine before being returned to 37°C for different periods of chase. When BFA was added, it was present at 10 μg/ml and was diluted from a 10-ng/ml stock made up in absolute ethanol and stored at −20°C. At the end of the experiments, cells were washed three times in HBSS, scraped into a conical 15-ml tube, centrifuged, and resuspended in 1 ml of 0.25 M sucrose, 20 mM Hepes, pH 7.4, containing 0.1-0.2 U of aprotinin. Cells were kept on ice for 10 min before homogenization with the A pestle of a Dounce homogenizer (Kontes Glass Co., Vineland, NJ). The number of strokes was adjusted so that about 80% of the cells were broken, as determined by trypan blue staining. The homogenate was centrifuged for 10 min at 1,000 rpm in a tabletop centrifuge (Beckman Instrs., Inc., Fullerton, CA), and the membrane present in the supernatant were pelleted at 75,000 rpm for 15 min at 4°C in the TLA10 rotor of a Beckman Instrs., Inc. Optima TLX ultracentrifuge. Pellets were dissolved in 100 μl of a phosphate buffered solution (1.5 mM KH2PO4, 8 mM Na2HPO4, 2H2O, 2.7 mM KCl, 137 mM NaCl) containing 1% SDS, 5 mM EDTA, 1% PMSF, and 0.2 mM NEM from a fresh stock. The solubilized membrane pellet was then diluted in 400 μl of the same phosphate buffered solution containing the same protease inhibitors and 1.25% Triton X-100. Two 5-μl aliquots of the solubilized membrane preparations were precipitated with trichloroacetic acid for determination of the incorporated radioactivity. Immunoprecipitations were performed on identical amounts of incorporated radioactivity with 0.5 μl of both the carboxy-terminal and exoplasmic loop antibodies, for 16-18 h at 4°C. Immunocomplexes were collected with protein A-Sepharose and washed once with each of the following solutions: (a) 20 mM Tris-HCl, pH 8.1, 1 M NaCl, 1% Nonidet P-40, 1 mM EDTA, 1% deoxycholate; (b) 20 mM Tris-HCl, pH 8.1, 1 M KCl, 0.1% Triton X-100; (c) 20 mM Tris-HCl, pH 8.1, 0.1% Triton X-100, 0.5% SDS, 0.5% deoxycholate; and (d) 20 mM Tris-HCl, pH 8.1, 0.2% Nonidet P-40. The protein A-Sepharose beads were then resuspended in Laemmli sample buffer (Laemmli, 1970) containing 5% SDS and loaded on 7.5% SDS-polyacrylamide gels. The gels were then treated for fluorography with diethylpyrocarbonate as described, washed in water, dried, and exposed to Kodak X-AR 5 films at −70°C for 3-7 d.

Glycosidase Treatments

Endo-β-glycosaminidase-H treatment was performed on immunoprecipitated GLUT2 as follows. After immunoprecipitation and washing of the immunocomplexes, a final wash was performed in 50 mM Na citrate, pH 5.5. The immunocomplexes were then incubated in the same citrate buffer with the enzyme for 18 h at 37°C before addition of Laemmli sample buffer and separation by gel electrophoresis. For treatment with neuraminidase, the immunocomplexes were washed as above, washed in digestion buffer (10 mM Tris-HCl, pH 6.0, 150 mM NaCl, 10 mM CaCl2) and then incubated in 500 μl of the same buffer with 0.1 U of enzyme for 30 min at 37°C before addition of Laemmli buffer and analysis by gel electrophoresis.

Results

Trypsin Sensitivity Assay

In freshly isolated islets GLUT2, most of the time, appears as multiple bands, the highest migrating with an apparent molecular mass of 62 kD (for example see Thorens et al., 1992) (data not shown). After an overnight period in culture medium GLUT2 is, however, detected as a single ~62-kD protein.
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**Figure 1.** Concentration-dependence of GLUT2 proteolytic cleavage by trypsin. Islets incubated overnight in culture medium were exposed to the indicated concentrations of trypsin at 4°C for 30 min before analysis of GLUT2 by immunoblotting. Total conversion of GLUT2 in a cleaved form is achieved with 10 μg/ml.

**Figure 2.** Determination of the trypsin cleavage site. Islets incubated overnight in culture medium or INS-1 insulinoma cells were treated with 20 μg/ml trypsin at 4°C for 30 min, lysed, and GLUT2 detected by immunoblotting with an antibody specific for the carboxy-terminal end of GLUT2 (C-term) or specific for amino acids 45–62 present in the first exoplasmic loop of GLUT2 (Exo). The immunoblots indicate that both the intact and cleaved forms of the transporter are recognized by the carboxy-terminal antibody but that the cleaved form has lost the epitope recognized by the exoplasmic loop-specific antibody. On the right part of the figure, a scheme of GLUT2 describes the regions of the transporter recognized by the two antibodies (heavy segments), the N-glycosylation site (N) and the sites of proteolytic cleavage by trypsin (arrows).

**Figure 3.** Time course of GLUT2 surface reexpression after trypsin cleavage. (A) Islets incubated overnight in culture medium were treated or not (C) with trypsin at 4°C for 30 min and either lysed directly or returned to 37°C for the indicated periods of time (hours). Islets were then lysed and GLUT2 analyzed by immunoblotting. (B) Densitometry scanning analysis of the kinetics of disappearance of cleaved GLUT2 (t1/2 = 2 h) and reexpression of GLUT2 on β cells membrane (t1/2 = 7 h). This experiment is representative of five experiments.

GLUT2 generated by trypsin digestion was detected only by the carboxy-terminal antibody but not by the antibody to the exoplasmic loop, demonstrating that this epitope was removed by the protease digestion. Identical results were obtained with GLUT2 expressed on the cell surface of either pancreatic β cells or INS-1 rat insulinoma cells (Fig. 2). The N-linked oligosaccharide is however still present on the cleaved form of the transporter as the deglycosylated form of noncleaved GLUT2 (see below) migrated with a faster mobility than the cleaved form. Therefore, protease digestion of GLUT2 took place in the exoplasmic loop, on the amino terminal side of the N-glycosylation site. The amino acid sequence of this region indeed shows the presence of multiple potential cleavage sites for trypsin, chymotrypsin, or elastase (Thorens et al., 1988).

**Surface Reexpression of GLUT2 in Pancreatic β Cells**

The trypsin sensitivity assay was used to determine the halftime of surface reexpression of GLUT2 after proteolytic cleavage of the transporter. Pancreatic islets were treated with trypsin at 4°C and then transferred back to 37°C for different periods of time before cell lysis and Western blot analysis of GLUT2 with the carboxy-terminal antibody. The disappearance of the cleaved form occurred with a t1/2 of 2–3 h and surface reexpression of GLUT2 at the cell surface was completed with a t1/2 of about 6–7 h (Fig. 3 A and B). The faster rate of disappearance of cleaved GLUT2 compared to the rate of reexpression suggested that once the structure of the transporter was modified, this induced a fast degradation of the protein.

**Biosynthetic Experiments**

For biosynthetic studies, we used the rat insulinoma cell line INS-1 which, unlike most insulinoma cell lines described so far, expresses a level of GLUT2 comparable to that of β cells and no GLUT1, even at passages >100 (this paper and data not shown). The use of pancreatic β cells for biosynthetic studies would require a very high number of isolated islets which would be extremely difficult to obtain to perform such experiments. Pulse labeling with [35S]methionine was, in all
The mature form of GLUT2 is in the TGN. INS-1 cells were pulse-labeled for 5 min and then chased for the indicated periods of time (min) before immunoprecipitation of GLUT2. The immunoprecipitates were then incubated in the presence or absence of neuraminidase. The increased mobility of the mature form of GLUT2 upon neuraminidase treatment indicated that this form had already acquired sialic acid, an event taking place in the TGN.

To assess whether the mature form of GLUT2 was already expressed on the surface, INS-1 cells were pulse-labeled for 5 min and subsequently chased in cold medium for increasing periods of time. At the end of the chase periods, cells were incubated on ice in the presence or absence of trypsin for 30 min. After washings in the presence of excess cold methionine, GLUT2 was then immunoprecipitated and separated on 7.5% polyacrylamide gels. The lower arrowhead shows the position of core-glycosylated GLUT2 (55 kD), the upper arrowhead shows the position of the fully glycosylated GLUT2 (62 kD). (B) Same experiment as in A but the immunoprecipitates were treated with or without endo-β-galactosaminidase H (endo-H). The coreglycosylated form of GLUT2 (upper left arrow) is converted into a faster migrating band (molecular mass = 53 kD) (lower left arrow) by glycosidase treatment. The mature form (right arrow) is insensitive to the enzyme.

Figure 4. Biosynthetic labeling of GLUT2 in INS-1 cells. (A) Insulinoma cells were pulse labeled for 5 min (5P) with [35S]methionine and then chased for the indicated periods of time (min) in the presence of excess cold methionine. GLUT2 was then immunoprecipitated and separated on 7.5% polyacrylamide gels. The lower arrowhead shows the position of core-glycosylated GLUT2 (55 kD), the upper arrowhead shows the position of the fully glycosylated GLUT2 (62 kD). (B) Same experiment as in A but the immunoprecipitates were treated with or without endo-β-galactosaminidase H (endo-H). The coreglycosylated form of GLUT2 (upper left arrow) is converted into a faster migrating band (molecular mass = 53 kD) (lower left arrow) by glycosidase treatment. The mature form (right arrow) is insensitive to the enzyme.

Figure 5. The mature form of GLUT2 is in the TGN. INS-1 cells were pulse-labeled for 5 min and then chased for the indicated periods of time (min) before immunoprecipitation of GLUT2. The immunoprecipitates were then incubated in the presence or absence of neuraminidase. The increased mobility of the mature form of GLUT2 upon neuraminidase treatment indicated that this form had already acquired sialic acid, an event taking place in the TGN.

Figure 6. Time course of GLUT2 transport from the TGN to the plasma membrane. (A) INS-1 cells were pulse-labeled for 5 min (5P) and chased in cold medium for the indicated periods of time (min). At the end of the chase periods, the cells were treated or not with trypsin at 4°C for 30 min before cell lysis and GLUT2 immunoprecipitation. Arrowhead on the left: coreglycosylated form of GLUT2; upper right arrowhead: mature form of GLUT2; lower right arrowhead: trypsin-cleaved form of surface-expressed GLUT2. Immunoprecipitation efficiency of trypsin-cleaved GLUT2 is reduced compared to immunoprecipitation of intact GLUT2 because the epitope recognized by the exoplasmic loop antibody (one of the two antibodies used for immunoprecipitation) is lost after trypsin digestion. (B) Densitometry scanning analysis of disappearance of coreglycosylated GLUT2, of appearance of the mature transporter and of its cell surface expression as assessed by acquisition of trypsin sensitivity. The mature form appeared with a t½ of 15 min and the t¼ of 15 min and the t½ for surface expression was 30 min indicating that GLUT2 transport from the TGN to the cell surface was achieved with a t½ of 15 min.
ence of trypsin inhibitors, the cells were lysed and GLUT2 immunoprecipitated for separation by gel electrophoresis. GLUT2 sensitivity to trypsin, as assessed by a shift in its electrophoretic mobility, was observed with a delay compared to appearance of the mature form (Fig. 6 A). Quantitative analysis by densitometric scanning of the autoradiograms (Fig. 6 B) indicated that the half-time for GLUT2 transport from the endoplasmic reticulum to the TGN was 15 min and that expression of the mature (sialylated) form of GLUT2 at the plasma membrane took place with a half-time of 30 min.

**Brefeldin A Inhibition of Intracellular Transport**

Altogether, the above experiments showed that GLUT2 intracellular transport to the plasma membrane follows the constitutive pathway of B cells. Moreover, our pulse–chase experiments indicated that biosynthetically labeled GLUT2 could be transiently accumulated in the TGN before being expressed at the cell surface. The amount of GLUT2 in the TGN at the steady state represents only a fraction of the total pool of GLUT2: GLUT2 residence time in the TGN is \( \tau_{1/2} \) of about 15 h for refilling the surface GLUT2 pool. Therefore at any given time, no more than a few percent of the transporter is in the TGN. By immunofluorescence microscopy, no internal GLUT2 could be demonstrated (not shown).

As the TGN is where the separation between the constitutive and regulated pathways occurs (Griffiths et al., 1986), we were interested in determining whether the exit from this compartment towards the plasma membrane via the constitutive pathway could be functionally distinguished from the regulated pathway. INS-1 cells were pulse labeled and chased for 30 min in the presence of BFA. This led to GLUT2 being retained as a 55-kD form which did not undergo further maturation to the 62-kD form (Fig. 7) or only partial modification with longer time of chase in the presence of BFA (see Fig. 8). These observations were consistent with a number of published works (Misumi et al., 1986; Doms et al., 1989; Lippincott-Schwartz et al., 1989) showing a partial and a slower rate of processing of proteins retained in the endoplasmic reticulum in the presence of BFA. We next pulse labeled the cells for 5 min and chased them for 15 min, a condition that allows the transient accumulation of a fraction of GLUT2 in the TGN, as described above (Figs. 7 and 8). BFA was then added and the cells chased for an additional 45 min before treatment of the cells in the presence or absence of BFA at 4°C. In cells incubated in the absence of BFA, a complete cleavage of GLUT2 was observed (Fig. 8) while the presence of BFA in the chase period prevented the acquisition of trypsin sensitivity by GLUT2.

A similar observation was made when the chase in the presence of BFA was for 2 h instead of 45 min (not shown). These observations indicated that a BFA-sensitive step was present in the transport of this protein between the TGN and the plasma membrane. We further showed that the block by BFA was reversible since washing the cells free from the drug followed by incubation of cells at 37°C for 1 h, led to complete recovery of GLUT2 trypsin sensitivity (Fig. 9).

A low temperature (20°C) block for intracellular transport of viral glycoproteins from the TGN to the plasma membrane has been described (Matlin et al., 1983; Saraste et al., 1984; Griffiths et al., 1985). To determine where the BFA block was localized relative to this temperature block, we

Figure 7. Brefeldin A blocks GLUT2 endoplasmic reticulum to Golgi transport. INS-1 cells were pulse-labeled for 5 min (P) and chased for 30 min in the presence or absence of 10 μg/ml BFA. In the absence of BFA, GLUT2 was converted in the mature form within the chase period. In contrast, in the presence of BFA, no maturation of GLUT2 was observed, indicating that GLUT2 had not been transported to the Golgi complex. The lower two bands detected below coreglycosylated GLUT2 were sometimes observed in pulse-chase experiments (see also Fig. 4 A).

Figure 8. Brefeldin A prevents GLUT2 transport from the TGN to the plasma membrane. INS-1 cells were pulse-labeled for 5 min (SP) and then chased for 15 min to allow accumulation of \( \sim 50\% \) of GLUT2 in the TGN. BFA was then added at 10 μg/ml and the cells chased for an additional 45 min. In the absence of BFA, GLUT2 becomes sensitive to trypsin, indicating that it was expressed at the cell surface. In the presence of BFA, the mature form of GLUT2 never became trypsin sensitive, indicating that it was retained intracellularly. The appearance of the immature form of GLUT2 as multiple bands in the presence of BFA may be due to partial processing of the oligosaccharide chain consequent to the mixing of the Golgi and ER compartments.

Figure 9. BFA block in transport from TGN to plasma membrane is reversible. INS-1 cells were pulse-labeled for 5 min, chased for 15 min, and exposed to BFA for an additional 45 min. This prevented surface expression of GLUT2 as assessed by trypsin sensitivity (lanes 1 and 2). Some cells were then washed free from BFA and the incubation was continued for an additional hour before treatment with or without trypsin and immunoprecipitation of GLUT2. GLUT2 sensitivity to trypsin was fully restored in the absence of BFA, indicating a complete reversibility of the block.

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GLUT2 could be measured (lanes 1 and 2) but all of the GLUT2 was in a mature form. Cells were then exposed (lanes 3 and 4) or not (lanes 5 and 6) to BFA for 15 min before the end of the low temperature incubation and further incubated for 2 h at 37°C and then treated (lanes 3 and 5) or not (lanes 4 and 6) with trypsin. In the absence or presence of BFA, GLUT2 acquired complete trypsin sensitivity to trypsin, indicating that the BFA block is proximal to the low temperature block.

After trypsin digestion, the cleaved transporter was very rapidly degraded with a t½ of ~2 h. Surface reexpression of newly synthesized GLUT2 was completed with a slower kinetics (t½ ~7 h). Since at the steady state the kinetics of reexpression equals the kinetics of degradation, the present protocol for measuring GLUT2 reexpression permits us to measure the turnover of the transporter on the plasma membrane. This protocol should also be useful to determine whether this parameter is modified in conditions of altered GLUT2 expression, such as in islets from diabetic animals.

Our data demonstrated that intracellular transport of GLUT2 to the β cell surface follows the constitutive pathway. Transport from the endoplasmic reticulum to the TGN proceeded with a half-time of ~15 min and surface expression from the TGN occurred with a similar half-time of ~15 min. Interestingly, we showed that BFA, a fungal metabolite, in addition to blocking endoplasmic reticulum to Golgi transport, could also block GLUT2 exit from the TGN.

BFA has been shown to interfere with the anterograde transport of proteins from the endoplasmic reticulum to the Golgi complex but to allow the retrograde transport of Golgi resident proteins including glycosyltransferases from the cis-, medial-, and trans-Golgi stacks to the endoplasmic reticulum (Doms et al., 1989; Lippincott-Schwartz et al., 1989; Lippincott-Schwartz et al., 1990; Klausner et al., 1992). This block of anterograde transport in the presence of continuous retrograde transport induces mixing of the Golgi complex with the endoplasmic reticulum. This could explain the partial processing of GLUT2 observed at longer periods of chase in the presence of BFA. However, no retrograde transport to the endoplasmic reticulum of TGN-specific proteins such as sialyltransferase (Chege et al., 1990) or TGN38 (Reaves et al., 1992) could be observed. In the case of TGN38, BFA has instead been shown to induce the relocation of this protein close to the microtubule organizing center, indicating that the TGN collapsed close to this structure. These and related observations (Lippincott-Schwartz et al., 1991) have led to the conclusion that the TGN and the bulk of the Golgi complex were separate membrane systems. In addition, TGN in the presence of BFA forms mixed tubular structures with the endosomal system (Lippincott-Schwartz et al., 1991; Wood et al., 1992). This mixed TGN/endosome compartment has, however, been shown to be able to allow continued internalization and recycling of membrane receptors to the cell surface.
GLUT2 exit from this structure in the presence of BFA therefore suggests that this transport step is carried out by vesicles requiring \( \beta \)-COP to bud from the TGN. Interestingly, when GLUT2 was first allowed to accumulate in the TGN at 22°C the addition of BFA before returning the cells to 37°C did not lead to a block of surface expression. This suggests that the low temperature block for exit from the TGN may be distal to the BFA block of vesicle budding.

In pancreatic \( \beta \) cells, the TGN is also the structure from which condensing insulin granules are formed. These are generated from budding, clathrin-coated, vesicles in which conversion of proinsulin to insulin is initiated (Orci et al., 1985, 1987). In the exocrine pancreas BFA was shown to have no effect on transport of proteolytic enzymes from Golgi complex to zymogen granules (Hendricks et al., 1992). Although, to our knowledge, no such evidence has yet been presented for pro/insulin segregation into condensing vacuoles, it is probable that clathrin-coated vesicle budding from the TGN towards the regulated secretory pathway is not impeded by BFA. Therefore, our study illustrates that in \( \beta \) cells, exit from the TGN towards the constitutive pathway is mediated by different transport mechanisms than regulated secretion.

In summary, as depicted in Fig. 11, we have demonstrated that GLUT2 was transported to the \( \beta \) cell surface via the constitutive pathway. This transport took place with a relatively fast kinetics. BFA blocked this transport at two different steps: at the exit from the endoplasmic reticulum and also at the exit from the TGN at a site proximal to the low temperature block. Once at the cell surface, GLUT2 resided permanently at the cell surface and did not recycle in intracellular vesicles. Cleavage of GLUT2 in the first extracellular domain with trypsin induced its rapid degradation and also allowed us to determine the kinetics with which surface reexpression of the intact transporter took place. The present description of the biosynthetic pathway for GLUT2 expression may permit a better understanding of the regulated expression of this transporter in glucose-unresponsive \( \beta \) cells. In particular, in situations in which GLUT2 decreased expression may result from translational or posttranslational alteration in its expression, as recently reported by Ogawa et al. in dexamethasone-induced diabetic rats (Ogawa et al., 1992).

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