This paper documents the effects of brefeldin A (BFA) on the processing and transport of viral envelope glycoproteins in a retrovirus-transformed murine erythroleukemia (MEL) cell line. BFA is a fungal metabolite that disrupts intracellular membrane traffic at the endomembrane reticulum (ER)-Golgi complex junction. In MEL cells, BFA inhibited the processing of the newly synthesized precursor, gPr90env, of the murine leukemia virus envelope protein, gp70, and curtailed the budding of virions into the culture medium by blocking the transport of this protein out of the ER. The block resulted in the intracellular accumulation of gPr90env and two putative products of its processing (78 and 66 kDa). The results of endoglycosidase (endo) H and D digestion of the viral glycoproteins in the presence and absence of BFA indicated that (i) there was no glycoprotein processing during the first ~2 h of the BFA block; (ii) active Golgi enzymes relocated to the ER in ~2 h during BFA treatment, resulting in the production of partially endo H-resistant forms of the spleen focus-forming virus glycoprotein, gp55 (in controls, this glycoprotein was generally retained in the ER as an endo H-sensitive entity); and (iii) proteolytic processing of gPr90env to gp70 occurred prior to the acquisition of endo H resistance and at approximately the same time as endo D sensitivity (i.e. in a cis Golgi compartment). In control cells, the gp55 focus-forming virus glycoprotein, gp55, underwent turnover with a half-life of ~5 h. In contrast, its turnover was considerably slower during BFA treatment ($t_{1/2} = 20$ h), suggesting that transport of gp55 out of the ER was required for its degradation or that BFA afforded it protection from proteolysis within the ER.

**EXPERIMENTAL PROCEDURES**

**Materials**—BFA was a generous gift from J. Lippincott-Schwartz and R. Klausner (National Institutes of Health [NIH]), and polyclonal goat anti-MuLV gp70 serum was kindly provided by S. Ruscetti (NIH). Recombinant endo β-N-acetylglucosaminidase H and Diplococcus pneumoniae endo β-N-acetylglucosaminidase D were from Boehringer Mannheim, and [$^{35}$S]methionine (1200 Ci/mmol) was from Amersham Corp. Cell Culture—MEL cells (clone 745) were grown at 37°C in a humidified atmosphere of 95% air, 5% CO$_2$ to densities ranging from 1 × 10$^5$ to 1 × 10$^6$ cells/ml in RPMI 1640 medium containing fetal bovine serum (10% v/v), 1-glutamine (0.3 mg/ml), penicillin (100 units/ml), and streptomycin (100 μg/ml). Cell viability was monitored by trypan blue exclusion.

**Metabolic Labeling**—For continuous metabolic labeling, cells were collected by centrifugation (10 min at 2000 × g), washed three times by resuspension-sedimentation in methionine-free RPMI 1640 medium, then incubated in the same medium (supplemented as mentioned above) containing 25 μCi/ml [$^{35}$S]methionine in the absence or presence of BFA at 1 μg/ml. In pulse-chase labeling experiments,
the cells were labeled as above, and an effective chase was obtained by adding a 750,000-fold excess of unlabeled methionine (final concentration of 1.5 mg/ml). In cases where recovery of MEL cells from BFA was investigated, the cells were removed from the culture medium by low speed centrifugation (10 min at 2,000 × g) and resuspended in complete RPMI 1640 supplemented as before, and further incubated for selected time periods. To end the labeling, the cells were collected by centrifugation and washed three times with ice-cold phosphate-buffered saline.

Isolation of Viruses—Metabolically labeled MEL cells were separated from the culture medium by low speed centrifugation (10 min at 2,000 × g). The ensuing supernatant was recovered and subjected to a second low speed spin to remove remaining cells or cell fragments, and then to high speed centrifugation (60 min at 40,000 × g) to recover shed virions. Coincident with shedding, the high speed supernatants were discarded, the pelleted virions were solubilized in 0.5% Nonidet P-40, 2% SDS, 10 mM dithiothreitol, 50 mM Tris-HCl, pH 7.5, and the ensuing lysates were processed through immunoprecipitation.

Immunoprecipitation and Gel Electrophoresis—Washed MEL cells (5 × 10^6/7 aliquot) were lysed for 15 min at 4°C in 0.5% Nonidet P-40 in 50 mM Tris-HCl, pH 7.5. Insoluble residues were removed by centrifugation (5 min at 13,000 × g). The resulting supernatants were mixed with 0.5 ml of 4% SDS, 20 mM dithiothreitol in 50 mM Tris-HCl, pH 7.5, and boiled for 10 min. After cooling to room temperature, the samples were processed for immunoprecipitation by the successive addition of (i) 50 ul of 1 M iodacetamide for 30 min at 37°C, (ii) 0.5 ml of 20% Triton X-100 for 15 min at 4°C, (iii) 3-5 µl of polyclonal goat anti-MuLV gp70 serum (1:300 to 1:500 dilution) for 15 min at 4°C, and (iv) 10 ng of Protein A-Sepharose CL-4B for 45 min at 4°C (with gentle agitation) to bind and recover antigen-antibody complexes. The beads were washed with 150 mM NaCl, 5 mM EDTA, 0.5% Nonidet P-40 in 50 mM Tris-HCl buffer, pH 7.5, (×1 min), then with the same buffer containing 500 mM NaCl (×1 min), and finally with distilled water (×1 min). Immunoprecipitates were released from the beads by boiling for 5 min with 3% SDS, 1.5% β-mercaptoethanol, 2 mM urea, 2 mM EDTA in 62.5 mM Tris-HCl buffer, pH 6.8. Samples of the ensuing lysates were adjusted to 5% glycerol and 10 µg/ml Pyronin Y and processed through a second low speed spin to remove remaining cells or cell fragments, and then the infected radioactivity was determined by fluorography of SDS-PAGE (as in Bonner and Laskey, 1974) and then dried. Radioactivity in individual proteins was estimated densitometrically using a Kodak Visage 2000 image analyzer or by liquid scintillation spectrometry of gel slices as previously described by us (Ulmer and Palade, 1989b) of the procedure of Cleveland et al. (1977).

Determination of Radioactivity—To assay [35S]methionine incorporation into total MEL cell proteins, aliquots (2 µl) of culture medium or cells in suspension were spotted onto Whatman 3MM filter paper, dried, and incubated in 10% trichloroacetic acid (total volume of 10 ml/filter) for 1 h at 4°C. The filters were rinsed in 5% sodium citrate, pH 6.5, for treatment with endo-6-N-acetylglucosaminidase H (endo H) digestion, and with 0.1% SDS, 0.7% Triton X-100 for 15 min at 4°C and then dried. Radioactivity was determined by fluorography of SDS-PAGE (as in Bonner and Laskey, 1974). Radioactivity in individual proteins was estimated densitometrically using a Kodak Visage 2000 image analyzer or by liquid scintillation spectrometry of gel slices as previously described by us (Ulmer and Palade, 1989b).

Glycosidase Digestion—Immunoprecipitated MuLV and SFFV glycoproteins were released from the Protein A-Sepharose beads by boiling for 10 min with 0.1% SDS and 1 mM phenylmethylsulfonyl fluoride in 1.5 M sodium citrate, pH 5.6, for endo-β-N-acetylglucosaminidase H (endo H) digestion, and with 0.1% SDS, 0.7% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 5 mM EDTA in 50 mM sodium citrate, pH 6.5, for treatment with endo-β-N-acetylglucosaminidase D (endo D). Samples (25 µl) were incubated with or without 2 µl of endo H or 2.5 µl of endo D for 16 h at 37°C and then solubilized as previously described, for subsequent SDS-PAGE and fluorography.

RESULTS

Effects of BFA on Protein Synthesis and Release into the Culture Medium

In MEL cells, [35S]methionine labeling of total acid-precipitable material was progressively stimulated by BFA so that, by 6 h, cell radioactivity was ~3-fold higher than in controls (Fig. 1A). This result, which may reflect intracellular accumulation of proteins as well as an increase in the rate of protein synthesis, is a departure from results obtained with other cell types (Misumi et al., 1986; Kato et al., 1989) in which protein synthesis was not found to be affected by BFA.

The total amount of acid-precipitable radioactivity released into the culture medium increased with time but remained relatively small and not strikingly different from controls (Fig. 1B). However, given the difference in [35S]methionine incorporation already mentioned, it amounted to ~10% of cell radioactivity in BFA-treated cells past 2 h, as opposed to ~27% in corresponding controls (Fig. 1C). Removal of BFA led to prompt increases in amounts (Fig. 1B) and percentage (Fig. 1C) of released acid-precipitable radioactivity. On a percentage basis, the latter reached initial levels over a 2-4 h period of recovery (Fig. 1C). Therefore, BFA leads to accumulation of newly synthesized proteins in MEL cells and decreases, but does not abolish, the release of such proteins into the culture medium.

Release of virions by MEL cells was investigated by virus isolation from culture media and immunoprecipitation of viral glycoproteins therefrom. In controls, radiolabeled gp70 was detected in post-assembly isolated from the culture medium by 2 h of continuous labeling (Fig. 2). In contrast, labeled gp70 was not detected in equivalent preparations from BFA-treated cells even after 6 h of continuous labeling, indicating that budding of virions containing newly synthesized coat proteins was abolished by the drug.

Effect of BFA on MuLV Glycoprotein Transport and Processing

gPr90kDa—the MuLV env gene encodes a precursor protein, gp90kDa, that gives rise to two components of the virus coat (gp70 and p15) by proteolytic cleavage assumed to occur late in the ER or early in the Golgi complex (Witte and Wirth, 1979; Fitting and Kabat, 1982). In control cells, cleavage of gp90kDa to yield gp70 was first detected by 30 min of continuous labeling (Fig. 3) and reached substantial levels past 2 h (Fig. 4). In a pulse-chase labeling experiment, gPr90kDa was mostly converted to gp70 by ~2 h of chase following a 1-h pulse (Fig. 5; see also Figs. 6 and 7). The level of labeled gp70 in the cells reached a maximum at ~1 h of chase and decreased gradually thereafter, suggesting that at this time protein was being shed by the cells, probably as assembled virions; the decrease coincides in time with the presence of detectable amounts of gp70 in released virions (see Fig. 2). As expected for a protein present in the ER, gPr90kDa accumulated in the presence of BFA, with no apparent conversion to mature gp70 (Fig. 4, see also Fig. 5B).

78 kDa—A protein of ~78 kDa was also immunoprecipitated from labeled MEL cells with the gp70 antisera (see Fig. 3 and open arrowhead on lane 1, Fig. 4). The 78-kDa protein displayed the following properties: (i) it is antigenically related to gPr90kDa and gp70 (it is immunoprecipitated by gp70 antisera); (ii) it was already metabolically labeled before 15 min, therefore prior to the appearance of labeled gp70 and at the same time or later than the detection of labeled gPr90kDa (Fig. 3); (iii) its radioactivity decreased with time during pulse-chase labeling in parallel with that of gPr90kDa (see Figs. 5–7); and (iv) it accumulated in BFA-treated MEL cells, presumably in the ER (see upper open arrowhead on lane 8, Fig. 4). With such properties, the 78-kDa protein could be a product of a distinct retrovirus related to MuLV and expressed in MEL cells or, more likely, a minor, transient intermediate in gPr90kDa processing.
Fig. 1. Effect of BFA on total protein synthesis and release into the culture medium. MEL cells were labeled with [³⁵S]Met for up to 6 h in the presence or absence of BFA (1 µg/ml), then recovered by sedimentation for further processing. In some cases, aliquots of cells taken at 2 h of incubation in the presence of BFA were resuspended in complete medium without BFA and further incubated for up to 4 h of chase/recovery (O). Acid-insoluble radioactivity was determined for cells in suspension and culture supernatant by trichloroacetic acid precipitation on filter paper (as described under “Experimental Procedures”), and plotted as total radioactivity in the cells (panel A), culture medium (panel B), and percent of total radioactivity (cells plus medium) recovered in the culture medium (panel C).

Fig. 2. Effect of BFA on the appearance of newly synthesized envelope proteins in budded virions. MEL cells were labeled with [³⁵S]Met for up to 6 h in the presence or absence of BFA (1 µg/ml), and budded virions, isolated from the culture medium by sedimentation, were processed through immunoprecipitation with anti-MuLV gp70 serum, SDS-PAGE, and fluorography. Numbers on lanes indicate h of incubation for control (no BFA) and BFA-treated cells.

Fig. 3. Metabolic labeling of MuLV and SFFV envelope proteins. MEL cells were labeled with [³⁵S]Met for 5, 15, 30, 60, and 90 min, sedimented, and processed through cell lysis, immunoprecipitation, SDS-PAGE, and fluorography. Numbers on lanes indicate min of labeling. Mobilities of SFFV gp55, MuLV gP50*, gp70, and a 78-kDa protein are indicated on the left side of the figure and those of molecular mass standards are given in kDa on the right side.

gp70 and 66 kDa—The appearance of labeled gp70 was completely inhibited by BFA, over the entire duration (6 h) of the experiments (Fig. 4). By 2-4 h in BFA, however, a protein of lower apparent mass than gp70 (~66 kDa) was immunoprecipitated from the cells (see lower open arrowhead on lane 8, Fig. 4). A similar protein was not detected in the absence of BFA in any experiments. This 66-kDa protein could be (i) a modified form of gp55; (ii) an experimental artifact (i.e. a degradation product of gp70, gP50*, or the 78-kDa protein); or (iii) a proteolytically processed form of
gPr90<sup>env</sup> or the 78-kDa protein. The latter possibility is the most likely explanation, based on the following observations: 
(i) the 66-kDa protein is more closely related in structure to gPr90<sup>env</sup>, gp70, and the 78-kDa protein than to gp55, as suggested by their susceptibility to trypsin and V-8 protease assessed by one-dimensional peptide mapping (not shown); 
(ii) there was apparently a precursor-product relationship between the 78- and 66-kDa proteins in BFA-treated cells (Fig. 5B); and 
(iii) labeled p15 appeared in BFA-treated cells at the same time as the 66-kDa protein (see lower panel, Fig. 4). In control cells, p15 appeared concomitantly with gp70, as expected, since it is the cleaved C-terminal segment of the MuLV gPr90<sup>env</sup> gene product. These results demonstrate that the proteolytic processing of gPr90<sup>env</sup> that yields gp70 is completely inhibited in the presence of BFA. Yet, a proteolytic event involving the MuLV envelope glycoproteins does occur after prolonged exposure to BFA (past 2 h), yielding a 66-kDa protein and p15. Under normal circumstances, the 66-kDa protein may exist only transiently or not at all, depending upon the temporal sequence of proteolytic cleavage and oligosaccharide processing (e.g. the 66-kDa protein may exist transiently if cleavage takes place before final oligosaccharide processing). In any case, these data demonstrate that proteolytic processing of virally encoded proteins does occur in the ER during BFA treatment, albeit slowly and incompletely, presumably due to relocation of Golgi protease(s) to the ER.

Effect of BFA on the Glycosylation of MuLV Envelope Proteins

During glycoprotein biosynthesis, asparagine-linked oligosaccharides are susceptible to digestion by endo H until converted from high mannose to hybrid or complex type by the action of enzymes in the middle and trans Golgi cisternal compartments (for review see Kornfeld and Kornfeld, 1985). In control cells, the MuLV-encoded glycoproteins, i.e. gPr90<sup>env</sup>, gp70, and the putative 78-kDa intermediate, were all endo H sensitive (Fig. 6A), indicating that proteolytic processing of gPr90<sup>env</sup> to gp70 occurs prior to the acquisition of endo H resistance (i.e. before middle to trans Golgi). Even after 5 h of chase, intracellular gp70 was mostly sensitive to endo H (Fig. 6A). Yet some gp70 molecules found in budded virions were completely endo H resistant (Geyer et al., 1984; see Fig. 6A), which demonstrates that up to 5 h a substantial portion of the newly synthesized gp70 molecules is retained in compartments prior to the middle Golgi and suggests that transit from the latter to the plasmalemma and virus budding therefrom are not the rate-limiting steps in the appearance of

![Diagram](image-url)
endoglycosidase H digestion of MuLV envelope proteins. MEL cells were labeled with [35S]Met for 1 h, followed by a chase period of up to 5 h, in the absence (panel A) or continuous presence (panel B) of BFA (1 μg/ml). In addition, MEL cells were continuously labeled for 8 h in the absence of BFA, followed by preparation of viruses from the culture medium (lanes 11 and 12; panel A). Aliquots of sedimented cells and viruses were processed through lysis and immunoprecipitation. The immunoprecipitates were incubated with (+) or without (-) endo H, and finally analyzed by SDS-PAGE and fluorography. Numbers on lanes indicate h of chase. In panel A, gPr90 and its endo H digestion product are marked by closed arrowheads in the (-) and (+) lanes, respectively. The 78 kDa and gp70 are indicated by open circles and open arrowheads, respectively in the (--) lanes; the same symbols are used in the (+) lanes to mark their endo H digestion products. In panel B, the same symbols are used for gPr90 and 78 kDa and their endo H digestion products. The dashed lines in lane 8 (+) mark partially endo H-resistant forms of the 78-kDa protein. The band moving faster than 78 kDa in lanes 2, 3, and 5 (--) is probably the 66-kDa protein.

FIG. 6. Endoglycosidase H digestion of MuLV envelope proteins. MEL cells were pulse-chase labeled and processed as for endo H digestion (see legend of Fig. 6 and "Experimental Procedures"). Open arrowheads in panel A (control cells) and panel B (BFA-treated cells) indicate the endo D-digested forms of gp70 and the 66-kDa protein, respectively. Labels on lanes indicate + or - endo D treatment and h of chase.

gp70 in virions in the culture medium. During BFA treatment, the 78-kDa protein became partially endo H resistant past 2 h of chase, presumably due to the relocation of Golgi enzymes to the ER (see dashed lines on lane 8, Fig. 6B). Endo D specifically removes N-linked oligosaccharides containing an α1-3-linked mannose residue unsubstituted in the 2 position (Mizouchi et al., 1984). Glycoproteins become endo D sensitive by the action of α1-2 mannosidase I in the cis Golgi compartment to yield the Man,GlcNAc₂ structure; subsequent addition of GlcNAc residues by GlcNAc transferase I in the middle Golgi compartment renders such proteins resistant to the enzyme once more (for review, see Kornfeld and Kornfeld, 1985). In both control and BFA-treated MEL cells, gPr90 and the 78-kDa protein were not discernably sensitive to endo D at any time during pulse-chase experiments (Fig. 7, A and B). In contrast, gp70 and a portion of the 66-kDa protein were reduced by ~2 kDa in apparent molecular mass by the glycosidase (see open arrowheads, Fig. 7, A and B). These results demonstrate that proteolytic processing of gPr90 to gp70 and the acquisition of endo D sensitivity by the latter were not resolved in time under our experimental conditions. Both apparently occur in a cis Golgi compartment. The endo D sensitivity of the 66-kDa protein probably reflects the redistribution of pertinent protease(s) and glycosyltransferases from the Golgi complex to the ER during BFA treatment.

Effect of BFA on SFFV gp55 Processing and Turnover

The structurally related gp55 is, for the most part, retained in the ER; only a small fraction is properly disulfide-bonded and transported to the cell surface (Gliniak and Kabat, 1989; Kilpatrick et al., 1989). In our experiments, gp55 accumulated in control MEL cells (no BFA) during continuous labeling experiments, likely due to its retention in the ER (Figs. 3 and 4). In pulse-chase experiments, however, labeled gp55 was lost with a half-life of ~5 h (Fig. 5C). This decrease is not likely to be due to processing and maturation, since the mature form of gp55, a 65-kDa protein (Ruta and Kabat, 1980), did not accumulate to a significant degree in these cells. A 65-kDa protein was immunoprecipitated from the cells after 4–6 h of labeling, but in negligible amounts by comparison with gp55 (see lanes 3 and 4, Fig. 4). It is more probable that this decrease in gp55 radioactivity represents protein degradation. Limited processing of gp55 occurred both in control and BFA-treated cells, as evidenced by a small but progressive decrease (up to ~1 kDa) in its mass during the chase period (see Figs. 3, 4, and 7), probably on account of trimming of glucose and some mannose residues in the ER (for review, see Kornfeld and Kornfeld, 1985). It is interesting to note that in the presence of BFA the amount of radioactivity in gp55 decreased only slightly during the chase period (t½ = ~20 h) (Fig. 5C), suggesting that the degradation of this protein is inhibited by BFA in the ER or requires its export out of the ER (e.g. to lysosomes).

Endoglycosidase digestion was performed on control and BFA-treated MEL cells to follow the redistribution of Golgi enzymes to the ER during BFA treatment; gp55, known to be retained in the ER, was used as a test object. In control cells, gp55 was completely sensitive to endo H for at least 5 h after its synthesis, resulting in a truncated form of ~45 kDa (see open arrowhead, Fig. 8A), which indicates that gp55, as expected, has only high mannose-type oligosaccharide chains.

FIG. 7. Endoglycosidase D digestion of MuLV and SFFV envelope proteins. MEL cells were pulse-chase labeled and processed as for endo D digestion (see legend of Fig. 6 and "Experimental Procedures"). Open arrowheads in panel A (control cells) and panel B (BFA-treated cells) indicate the endo D-digested forms of gp70 and the 66-kDa protein, respectively. Labels on lanes indicate + or - endo D treatment and h chase.

FIG. 8. Endoglycosidase H digestion of SFFV gp55. MEL cells were pulse-chase labeled and processed as described in the legend of Fig. 6. Open arrowheads in lanes A and B indicate the endo H-digested form of gp55 in control cells and the partially endo H-resistant forms of gp55 in BFA-treated cells, respectively. The nature of the protein resistant to endo H treatment migrating faster than gp55 on the gel is presently unknown.
In BFA-treated cells, however, gp55 gradually became partially resistant to endo H and, past 1 to 2 h of chase in the presence of BFA, incompletely digested forms of ~47, ~49, and ~51 kDa were observed (see open arrowheads, Fig. 6B). The presence of three intermediate forms of gp55 revealed by endo H digestion agrees in general with previous reports that this protein has 4 N-linked oligosaccharide chains (Polonoff et al., 1982; Srivinas and Comans, 1983).

**DISCUSSION**

The viral envelope glycoproteins synthesized in MEL cells provide a useful system to study intracellular protein transport and processing, since they are comprised by two structurally related gene products (encoded by two different viruses) that have distinct biogenetic fates. The first, the SFFV gp55 protein is, for the most part, not exported out of the ER, thereby providing an ER biochemical marker. The second, the MuLV envelope glycoprotein, is transported to the cell surface and in transit undergoes proteolytic and oligosaccharyl processing steps that can be used to define the kinetics of these steps, to follow the progress of the protein through the ER and Golgi complex and the redistribution of Golgi enzymes to the ER. Finally, intracellular virus particles and budded virions can be readily detected by electron microscopy. Hence, the effects of BFA can be easily and extensively monitored in this system.

**Effects of BFA on MEL Cell Protein Transport and Metabolism**—As with proteins in other cells (Takatsu and Tamura, 1986; Misumi et al., 1986; Kato et al., 1989; Oda et al., 1990), BFA curtailed without abolishing the release of newly synthesized MEL cell proteins into the culture medium. This continued appearance of labeled proteins in the culture medium in the presence of BFA was not due to an ineffective block in exocytosis by the drug, based on morphological and biochemical data (not shown). Rather, this may represent cytolysis or some other process that remains to be elucidated. In contrast, budding of virus containing newly synthesized coat proteins was completely inhibited by BFA. Yet, virions apparently continued to bud in the presence of the drug, as observed by electron microscopy (Ulmer and Paleade, 1991); their budding probably involved complete virus particles that were already past the Golgi complex at the time of BFA administration and/or viruses containing only the gag gene products (Shields et al., 1978). Further work using probes to other MuLV proteins will be necessary to determine the nature of these virus particles. These slow budding viruses are, however, a small minority, since the rate-limiting step in virus maturation seems to be in the Golgi complex or earlier, as evidenced by the accumulation of endo H- and D-sensitive forms of MuLV gp70 in control cells. Moreover, previous work on this system (Fitting and Kabat, 1982) and others (Williams et al., 1985) has demonstrated that exit of viral proteins from the ER seems to be the rate-limiting step in protein transport to the cell surface.

**General Effects of BFA on Viral Glycoproteins in MEL Cells**—BFA blocked transport of newly synthesized viral envelope proteins out of the ER to the Golgi complex in MEL cells and in addition caused a redistribution of Golgi enzymes to the ER. These changes in traffic patterns were documented primarily biochemically as the absence of Golgi-type modifications during the first 2 h and the late appearance of such modifications past 2 h. In our case, we had the advantage of an ER marker, gp55, that in controls remained permanently endo H sensitive, whereas under BFA it acquired endo H resistance past 2 h. In work done in other laboratories, the redistribution of Golgi antigens was also demonstrated by immunocytochemical tests (Lippincott-Schwartz et al., 1989; Domas et al., 1989). Our results are in agreement with data already reported on murine glycoporphins in MEL cells (Ulmer and Paleade, 1989a), the T cell antigen receptor in murine T cell hybridomas (Lippincott-Schwartz et al., 1989), and G proteins in vesicular stomatitis virus-infected baby hamster kidney cells (Takatsu and Tamura, 1985), Chinese hamster ovary cells (Doms et al., 1989), and hepatocytes (Oda et al., 1990).

**Specific Effects of BFA on Viral Glycoproteins in MEL Cells**—Besides these general changes, applicable to all cases studied to date, our results have revealed additional modifications that so far apply only to the viral proteins produced in MEL cells. First, the SFFV glycoprotein gp55, which turned over with a half-life of ~5 h in control cells, accumulated and turned over at a considerably slower rate (1/2 = ~20 h) in the presence of BFA. Therefore, either gp55 requires transport out of the ER for degradation or BFA inhibits its degradation within the ER. Further experiments will be required to determine whether gp55 is degraded in the lysosomes or in the ER. The latter site has recently been proposed in the disposal of vesicular stomatitis virus-infected baby hamster kidney cells (Takatsu and Tamura, 1985), Chinese hamster ovary cells (Doms et al., 1989), and hepatocytes (Oda et al., 1990).
gPr90\textsuperscript{wv} does not leave the ER (i.e. it is locally degraded). But, in the presence of BFA, the protease(s) involved in the production of gp70 and p15 from gPr90\textsuperscript{wv} relocates from the Golgi complex to the ER, where it can act on the 78-kDa protein to yield the 66 kDa truncated form of gp70.

Localizing Processing Events—The cleavage of gPr90\textsuperscript{wv} to gp70 and p15 appears to occur in the cis Golgi, based on the following lines of evidence: (i) gPr90\textsuperscript{wv} accumulated in the presence of BFA, with no evidence of cleavage to gp70; (ii) removal of BFA resulted in the appearance of gp70, concomitantly with the disappearance of gPr90\textsuperscript{wv}; (iii) gPr90\textsuperscript{wv}, gp70, and the two related proteins (78 and 66 kDa) were endo H sensitive, indicating that proteolytic processing of gPr90\textsuperscript{wv} to gp70 takes place before they reach the middle Golgi compartment, where endo H resistance is acquired; and (iv) gp90\textsuperscript{wv} and the 78-kDa protein were endo D resistant, whereas gp70 and the 66-kDa protein were sensitive to the glycosidase, suggesting that, in our experimental conditions, the cleavage event cannot be resolved in time from the acquisition of endo D sensitivity in all cases. It is believed that endo D sensitivity is conferred upon arrival in the cis Golgi compartment, when oligosaccharides are trimmed to the Man\textsubscript{5}GlcNAc\textsubscript{2} structure (for review, see Kornfeld and Kornfeld, 1985). Therefore, the cleavage of gp90\textsuperscript{wv} to gp70 must occur distal to the BFA block in MEL cells (i.e. past the ER) but not past the cis Golgi. The proteolytic processing of gPr90\textsuperscript{wv} to gp70 and p15 in an early Golgi compartment in MEL cells is in contrast to similar processing of the envelope proteins in other retroviruses, which takes place in a late Golgi compartment (for review, see Krausslich and Wimmer, 1988).

During BFA treatment, the 66-kDa protein appeared at the time when SFFV gp55 and the 78-kDa protein became past the cis Golgi compartment, when oligosaccharides are trimmed to the Man\textsubscript{5}GlcNAc\textsubscript{2} structure (for review, see Kornfeld and Kornfeld, 1985). Therefore, the cleavage of gp90\textsuperscript{wv} to gp70 must occur distal to the BFA block in MEL cells (i.e. past the ER) but not past the cis Golgi. The proteolytic processing of gp90\textsuperscript{wv} to gp70 and p15 in an early Golgi compartment in MEL cells is in contrast to similar processing of the envelope proteins in other retroviruses, which takes place in a late Golgi compartment (for review, see Krausslich and Wimmer, 1988).

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Kornfeld, R., and Kornfeld, S. (1985). Therefore, the cleavage of gp90\textsuperscript{wv} to gp70 must occur distal to the BFA block in MEL cells (i.e. past the ER) but not past the cis Golgi. The proteolytic processing of gp90\textsuperscript{wv} to gp70 and p15 in an early Golgi compartment in MEL cells is in contrast to similar processing of the envelope proteins in other retroviruses, which takes place in a late Golgi compartment (for review, see Krausslich and Wimmer, 1988).

SUMMARY

BFA has enabled us to study in detail several aspects of the processing of viral envelope proteins in MEL cells. First, a small fraction of the MuLV envelope protein gp90\textsuperscript{wv}, undergoes an apparent proteolytic cleavage in its N terminus while in the ER to yield a 78-kDa protein. Second, the normal proteolytic processing of gp90\textsuperscript{wv} that gives rise to gp70 and p15 apparently occurs in a cis Golgi compartment, unlike the cleavage of other retroviral envelope proteins which is effected in a trans Golgi compartment. Finally, SFFV gp55, which is for the most part not transported out of the ER to the Golgi complex, undergoes turnover at a rate that is substantially reduced by BFA. The site of gp55 degradation remains to be established.

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