Protoplasma (1993) 177: 15–22

PROTOPLASMA

© Springer-Verlag 1993
Printed in Austria

Low temperature compartment formation in feline immunodeficiency virus-infected and uninfected feline kidney cells

D. J. Morré* and **M. Paulik**

Department of Medicinal Chemistry, Purdue University, West Lafayette, Indiana

Received August 13, 1993
Accepted September 13, 1993

Summary. This study was to determine if feline immunodeficiency virus (FIV)-infected and uninfected Crandall feline kidney (CRFK) cells exhibited a low temperature (16 °C) block in membrane trafficking between transitional endoplasmic reticulum and Golgi apparatus represented by intermediate compartment formation. Cells were cultured at different temperatures and membrane changes involving the Golgi apparatus and Golgi apparatus-associated membrane structures were monitored by electron microscopy and quantitated. With 30 min of incubation, membranes of the Golgi apparatus stack increased in amount at temperatures of 16 °C and below compared to temperatures above 18 °C. The increase was greatest along the major polarity axis as evidenced by an increased stack height. Neither the number of cisternae per stack nor the average stack diameter (width) was affected by temperature. The response was maximal between 15 and 30 min of low temperature treatment of the cells. Results with cells infected and uninfected with feline immunodeficiency virus were similar. The increase in stack height was due primarily to an increase of membranes at the cis face (cis Golgi apparatus network). At 18 °C, membranes of the trans Golgi apparatus network accumulated suggesting that import from the cis Golgi network could proceed at this temperature, whereas exit from the trans Golgi network was still at least partially blocked. Also increased at 16 °C and below were numbers of transition vesicles in the space between the Golgi apparatus and the transitional endoplasmic reticulum associated with the cis Golgi apparatus face. The results suggested interruption of the orderly flux of membranes into the Golgi apparatus at 16 °C and below. Moreover, the block appeared to be reversible. Upon transfer from 16 °C to 37 °C, there was a time-dependent decrease in the accumulations of cis compartment membrane accompanied by a corresponding equivalent increase in the membranes of the trans Golgi apparatus compartment.

Keywords: Feline immunodeficiency virus; Golgi apparatus; Low temperature compartment; Trans Golgi network; Feline kidney cells.

* Correspondence and reprints: Department of Medicinal Chemistry, Hansen Life Sciences Research Building, Purdue University, West Lafayette, IN 47907, U.S.A.

Introduction

The feline immunodeficiency virus (FIV) has been proposed as a model for the human immunodeficiency virus (HIV) (Talbott et al. 1989). Our research was to use a cell-free system that reconstitutes membrane traffic between the transitional endoplasmic reticulum and the Golgi apparatus (Morré et al. 1986, Nowack et al. 1987, Paulik et al. 1988) to study factors that may influence trafficking and processing of the major FIV coat proteins. These studies would be facilitated by a reversible means to accumulate the viral glycoproteins in an unprocessed form in a pre-Golgi apparatus compartment. One approach to achieving such an accumulation would be to utilize low temperature (Tartakoff 1986). In pancreatic ascinar (Tartakoff 1986) and other cells (Lagunoff and Wan 1974, Holmes et al. 1981, Fries and Lindstrom 1986, Saraste et al. 1986), at temperatures of 16 to 18 °C or below, secretory proteins were blocked and accumulated in a pre-Golgi apparatus compartment. At 20 °C a medial Golgi apparatus compartment was reached (Saraste and Kuismanen 1984). Similar results were obtained with the intracellular transport and surface expression of proteins in several other virus-infected cell systems (Matlin and Simons 1983, Tooze et al. 1984, Tooze et al. 1988, Griffiths et al. 1985, Copeland et al. 1988).
In this report we quantitated the response of Crandall feline kidney (CRFK) cells to low temperature. The results showed a 16 °C block in the trafficking of membranes between the transitional endoplasmic reticulum and the Golgi apparatus that resulted in an accumulation of membranes in an intermediate compartment

at the cis face of the Golgi apparatus. The block in membrane traffic appeared to be nearly complete but reversible as the accumulated membranes and vesicles of the cis Golgi apparatus network were replaced by an equivalent accumulation of vesicles and membranes within 15 to 30 min upon transfer of cells from 16 °C to 37 °C.

Materials and methods

Cell culture

CRFK (Crandall feline kidney) cells were grown in D-MEM (Dulbecco's minimum essential medium) supplemented with 10% fetal calf serum, 1% MEM non-essential amino acids, 1% BME vitamins, 1% sodium pyruvate, 1% penicillin (100 U/ml), 1% streptomycin (100 µg/ml) and 50 mg gentamicin/liter.

Virus infection

Infected CRFK cells were maintained from a CRFK cell stock that was chronically infected with the Petaluma strain of FIV (Phillips et al. 1990).

Temperature incubations

All temperature experiments were carried out in constant temperature incubators (± 0.5 °C). The incubators were housed in a refrigerated room (4 °C) to ensure that the room temperature never exceeded that of the chambers. Equilibration of culture media and cells to the temperature of the chambers occurred rapidly in a matter of a few minutes. Fixations were at the same temperature as the incubations.

Electron microscopy

Cells or isolated fractions were fixed after temperature incubation in 2.0% glutaraldehyde in 0.1 M sodium phosphate, pH 7.2 followed by post fixation in osmium tetroxide in the same buffer. Dehydration

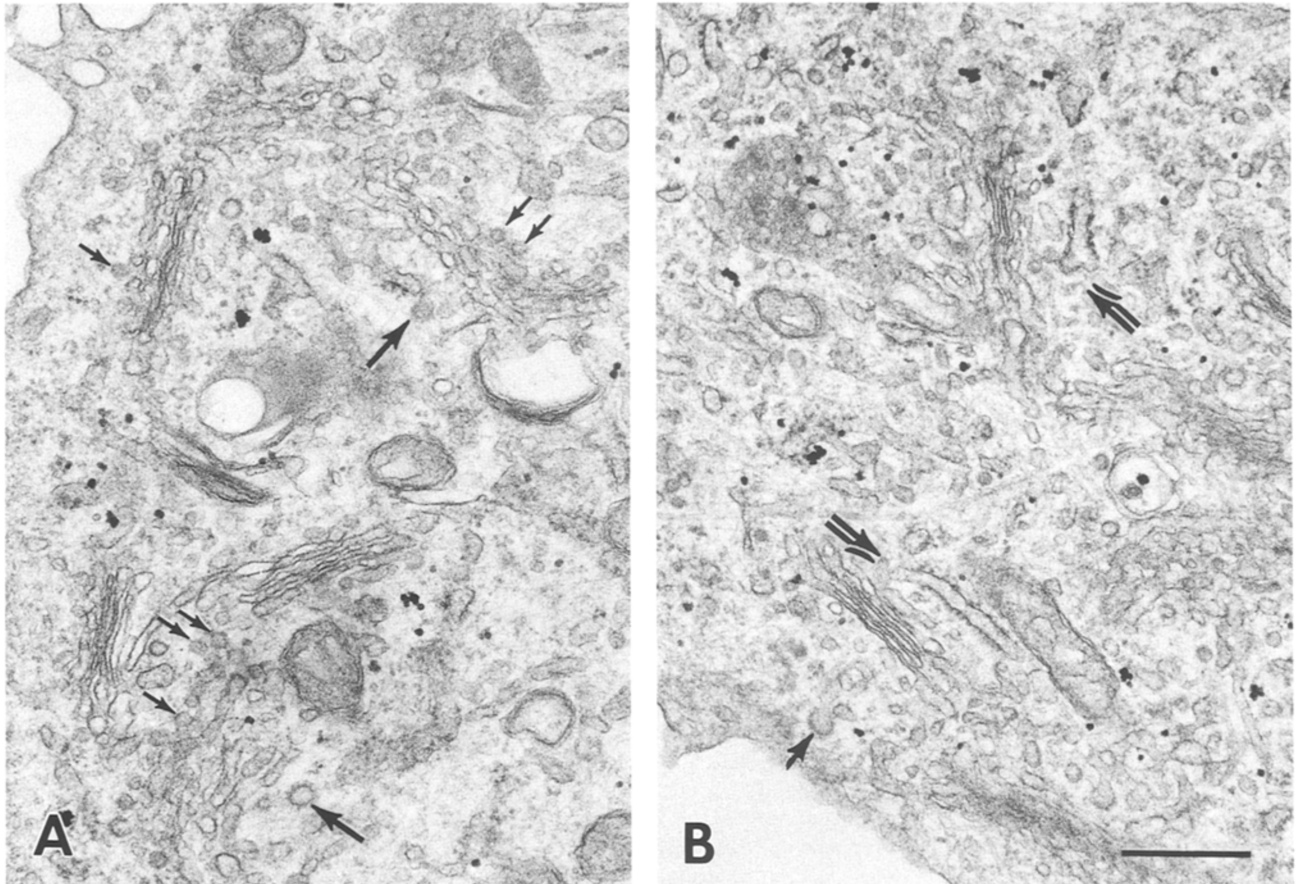

Fig. 1 A, B. Portions of uninfected CRFK cells illustrating the appearance of the Golgi apparatus at 37 °C. **A** The trans face and the trans Golgi network (TGN) were identified as those membranes distal to the aligned portions of the Golgi stack associated with clathrin-coated vesicles (large arrows). Irregular, often dilated and endoplasmic reticulum-associated membranes at the opposite Golgi apparatus face were designated the cis Golgi network (CGN). Putative 50 to 70 nm "transition" vesicles (small arrows) were located in this region but clathrin-coated vesicles were absent. **B** As in **A** except to more clearly illustrate the association of the cis Golgi network and budding profiles with the Golgi apparatus-associated transitional endoplasmic reticulum (double arrows). The ultrastructural appearance was unchanged by virus infection. Bar: 0.5 µm

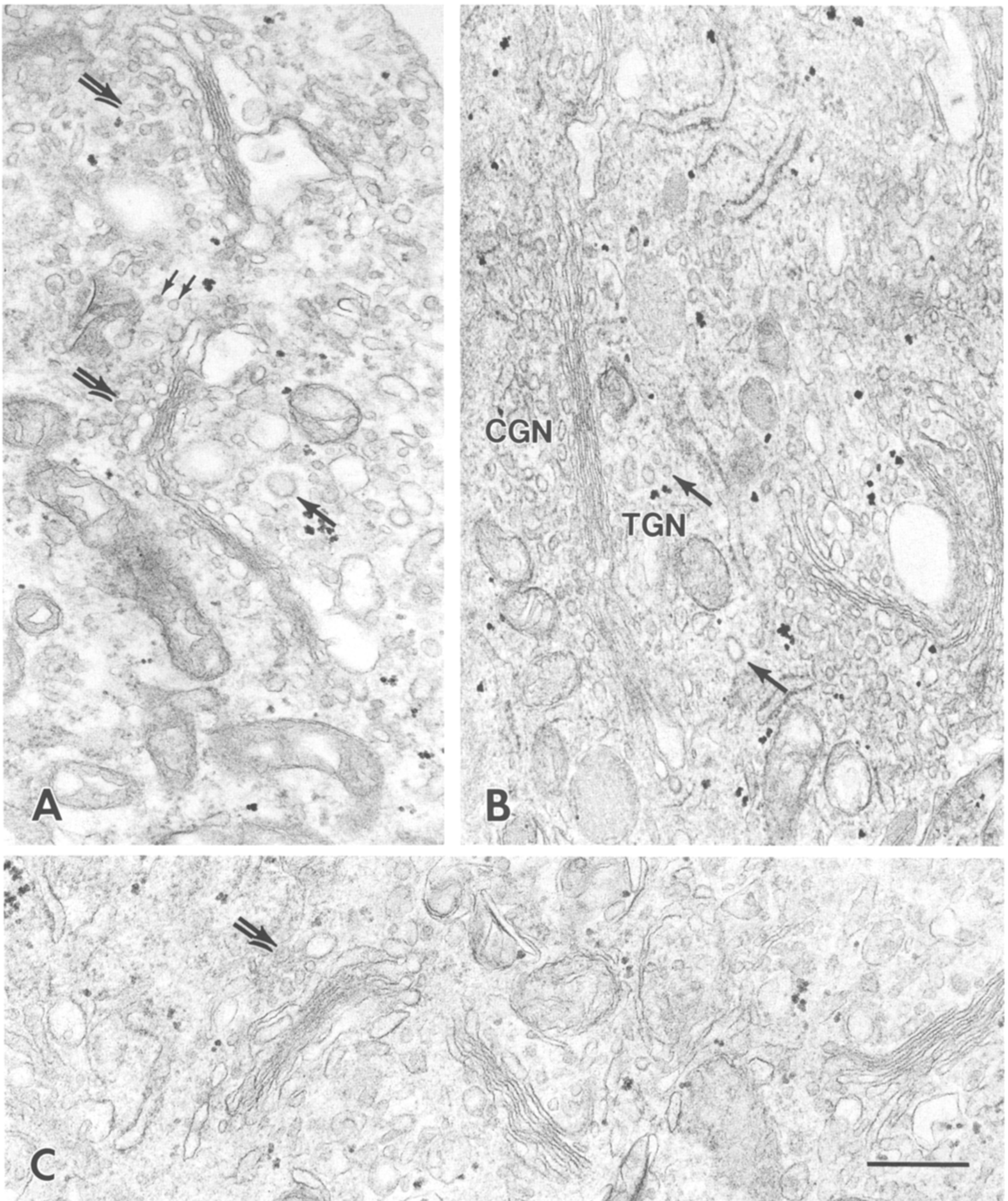

Fig. 2 A–C. Portions of uninfected CRFK cells after 30 min of incubation at low temperatures. **A** At 16 °C, the Golgi apparatus stacks became surrounded by more membrane material while the number of stacked cisternae remained constant. The increased membrane material was located largely at the cis Golgi apparatus face (double arrows) and consisted of irregular structures or vesicles, partially fused, with swollen interiors and morphologically distinct from the cisternal stacks. Vesicular profiles, 50–70 nm in diameter, representing putative transition vesicles (small arrows) were increased but did not accumulate. Clathrin-coated vesicles (large arrows) marked the trans Golgi network. **B** Infected cell incubated at 18 °C for 30 min. The membrane material of both the cis Golgi network (*CGN*) and the trans Golgi network (*TGN*) was increased. **C** Uninfected cell incubated for 30 min at 4 °C. A collection of partially budded transition vesicles is illustrated at the double arrows. Bar: 0.5 μm

was through an acetone series with embedment in Epon (Luft 1961). Thin sections were observed and photographed using a Philips EM 200 electron microscope.

Results

CRFK cells contained an extensive system of Golgi apparatus consisting of dispersed stacks of about 4 cisternae each (Fig. 1). On average the stacks were about 1 µm in diameter (width) with a height exclusive of the trans Golgi apparatus network and associated pre-Golgi apparatus vesicles of about 0.25 µm (Fig. 1). After 30 min incubation at different temperatures, a much more extensive Golgi apparatus was seen at 16 °C (Fig. 2 A, C) and below. The alteration was most evident as an increase in the height of the cisternal stacks (Fig. 3) as seen in both uninfected cells (Fig. 3 A, B) and cells infected with FIV (Fig. 3 C, D). The diameter of the stacks, on average, remained constant over the temperature range 4 to 37 °C as did the number of cisternae per stack (Fig. 3). In both uninfected (Fig. 3 A, B) and virus-infected (Fig. 3 C, D) cells, Golgi apparatus height nearly doubled by 15 to 30 min of incubation at 16 °C.

The basis for the progressive increase in stack height could not be found in an increase in the number of clearly defined cisternae (Figs. 2 and 3). The increase in height was due, rather, to an accumulation of irregular membranes consisting of closely packed and partially fused vesicles at the cis face of the Golgi apparatus stack (cis Golgi network) (Fig. 4). These elements often were swollen and were not organized morphologically into cisternae aligned with those of the existing stack (Fig. 2 A). Also during the first 10 min of low temperature incubation, the number of 50 to 70 nm vesicles of the cis Golgi apparatus face increased approximately 2-fold (data not shown). However, these vesicles represented less than 15% of the total accumulated membranes at the cis Golgi apparatus face.

To test the reversibility of the increase, cells incubated at 16 °C were returned to 37 °C for varying times up to 30 min. Surprisingly, the height of the Golgi apparatus did not return to control values as expected. Rather it remained constant (Fig. 5). The anomaly was resolved when the cis Golgi network and the trans Golgi network membranes were analyzed separately (Fig. 6). The trans Golgi network was identified by the presence of clathrin-coated vesicles. The cis Golgi network was identified as an irregular and slightly swollen system of cis-

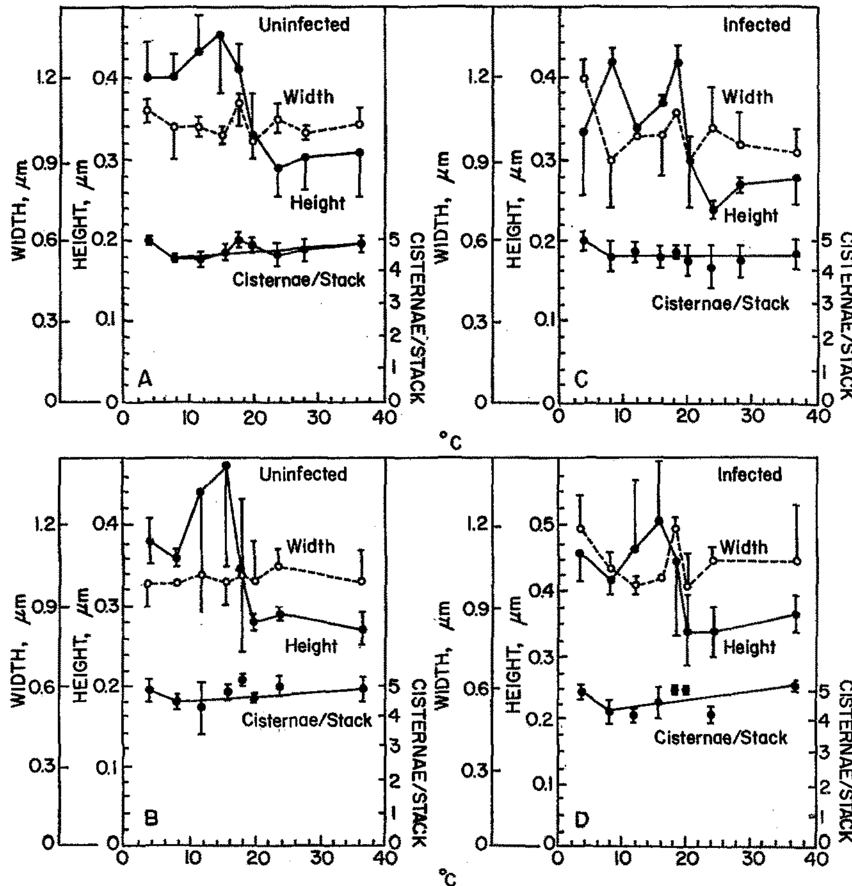

Fig. 3. Quantitation of the response of the Golgi apparatus of uninfected (**A, B**) and FIV infected (**C, D**) CRFK cells to increasing temperature over the range of 4 to 37 °C. Illustrated are stack width (an approximation of mean cisternal diameter), total stack height and the number of parallel, complete cisternae per stack. Each data point represents an average of 3 determinations from 10 Golgi apparatus stacks photographed at random ± standard deviations. **A** and **C** and **B** and **D** represent separate experiments with uninfected and FIV-infected cells incubated in parallel

ternae, vesicles and tubules and the absence of clathrin-coated vesicles at the opposite Golgi apparatus face (Fig. 7). With increasing time of incubation at 37 °C a decline in the cis Golgi membranes was accompanied by a corresponding and equivalent increase in trans Golgi apparatus membranes (Fig. 6). Total Golgi apparatus amount remained constant. However, during the recovery phase the relative amounts of cis Golgi apparatus membranes decreased and the trans Golgi apparatus membranes increased in a balanced inverse relationship.

Discussion

For cultured cells undergoing virus replication, a low-temperature compartment has been reported previously (Lagunoff and Wan 1974; Matlin and Simons 1983; Saraste and Kuismanen 1984; Tooze et al. 1984, 1988). Reduced temperature has been shown to block Golgi apparatus-mediated steps in post translational processing and secretion of proteins in tissues as well (Holmes et al. 1981, Brand et al. 1985, Fries and Lindstrom 1986, Saraste et al. 1986, Tartakoff 1986).

In liver slices, low temperature resulted in large accumulations of individual transition vesicles which formed but failed to fuse with the cis Golgi apparatus at temperatures of 16 °C and below (Morré et al. 1989). A low temperature block at 16 °C was observed for a green alga, *Micrasterias americana* (Noguchi and Morré 1991) as well.

In the CRFK cells investigated here, the morphological response of the Golgi apparatus and associated mem-

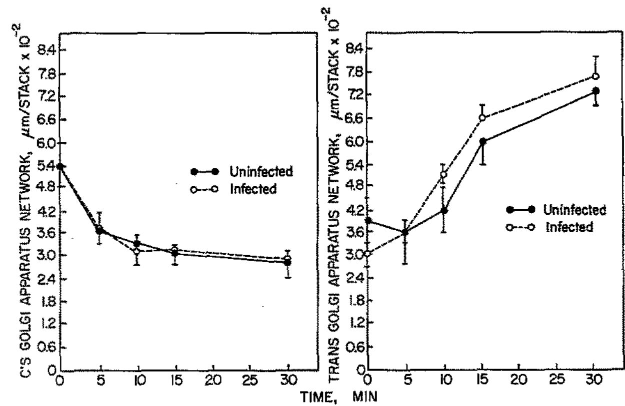

Fig. 6. Relative amounts of the cis Golgi network (CGN) and the trans Golgi network (TGN) per Golgi apparatus stack as a function of time after transfer from 16 °C to 37 °C. Uninfected or virus-infected cells were incubated for 30 min at 16 °C and then transferred to 37 °C to initiate the recovery phase. At the times indicated, cells were fixed for morphological analysis. Averages represent estimates from 3 determinations of 10 stacks each ± standard deviations

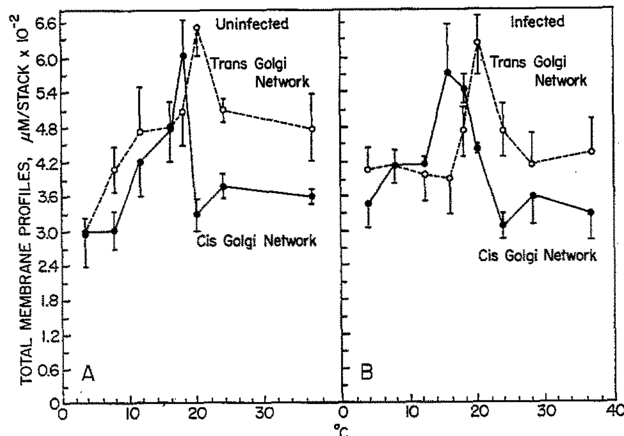

Fig. 4. Relative amounts of the cis Golgi network and the trans Golgi network per Golgi apparatus stack as a function of temperature. Uninfected or virus-infected cells were incubated for 30 min at the temperature indicated as for Fig. 3 after which cells were fixed for morphological analysis. Averages represent estimates from 3 determinations of 10 stacks each ± standard deviations

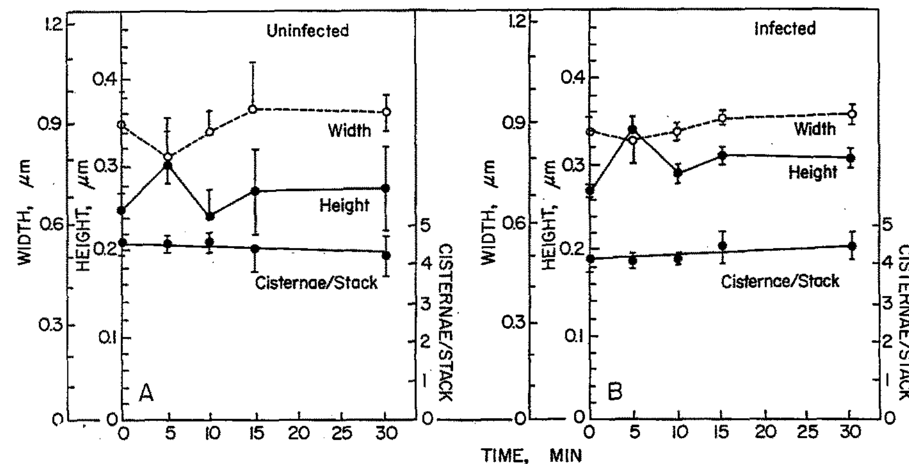

Fig. 5. Response of the Golgi apparatus of uninfected (**A**) and FIV-infected (**B**) CRFK cells to time of incubation at 16 °C comparing stack width, total stack height and number of parallel, complete cisternae per stack. Each data point represents 3 determinations from 10 Golgi apparatus stacks photographed at random ± standard deviations

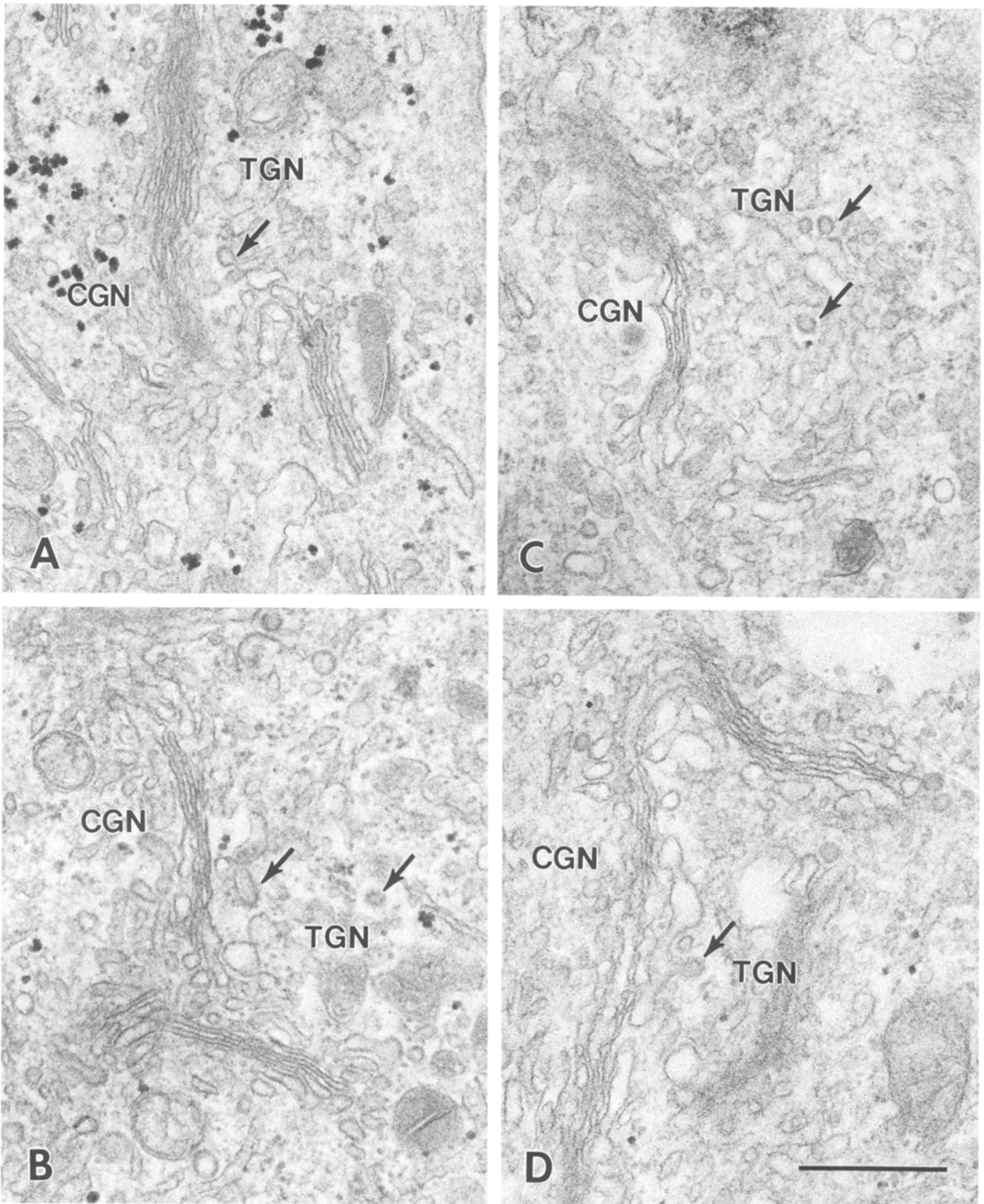

Fig. 7. Portions of uninfected CRFK cells incubated 30 min at 16 °C followed by transfer to 37 °C for 0 (**A** and **B**) or 30 min (**C** and **D**) to evaluate recovery. After incubation at 16 °C, the cis Golgi network (*CGN*) was well developed and the trans Golgi network (*TGN*) less so (**A** and **B**). By 30 min after transfer to 37 °C, the CGN was much reduced in amount, but replaced by a nearly equivalent amount of membranes at the TGN (**C** and **D**). This reciprocal relationship was quantitated and illustrated in Fig. 6. The TGN was identified as the region of the Golgi apparatus occupied by clathrin-coated membranes and vesicles (large arrows). Bar: 0.5 μm

brane systems to temperatures of 16 °C and below was comparable to that seen in other systems but with important differences. In contrast to results with liver slices and cultured chinese hamster ovary cells (Morré et al. 1989), transition vesicles accumulated, but most evident was the formation at the cis Golgi apparatus face of a smooth membrane compartment, endoplasmic reticulum-like in character, consisting of irregular tubules and partly fused vesicles. These structures, corresponding to a cis Golgi apparatus network (CGN), were in sharp contrast to those found in liver where vesicle integrity was maintained (Morré et al. 1989). The CGN was formed proximal to the location of transition vesicles such that its formation by fusion of the transition vesicles was regarded as unlikely. Rather it seemed to represent an accumulation of transitional endoplasmic reticulum membranes that failed to bud or where the buds had partially formed and failed to separate. At 18 °C, the CGN block was partly relieved and replaced by a comparable block at the opposite Golgi apparatus face that resulted in an accumulation of membranes of the trans Golgi network (TGN).

Progression from the trans Golgi network beyond the Golgi apparatus is known to be prevented at temperatures below 20 °C (Saraste et al. 1986). Under these conditions, processing events characterizing the medial Golgi apparatus compartment take place with glycoproteins transferred from endoplasmic reticulum. Yet these glycoproteins do not reach the cell surface indicative of a temperature block involving exit from the Golgi apparatus. This is evidenced as well in our studies, where the accumulations of trans Golgi apparatus material occurred at 18 °C and 20 °C whereas the cis Golgi apparatus accumulations were restricted to temperatures of 16 °C and below. In general, the responses of the Golgi apparatus and associated membranes to temperature were similar in uninfected and FIV-infected cells.

In liver, the transition vesicles that form and accumulate at low temperature (16 °C) do not appear to progress rapidly through the Golgi apparatus upon transfer to 24 °C or 37 °C (M. Paulik and D. J. Morré, unpubl. results). However, the cis Golgi compartment accumulated by the CRFK cells in response to a 16 °C temperature block appeared completely reversible. Whereas the membrane accumulations generated at 16 °C remained associated with the Golgi apparatus over 30 min, the accumulations disappeared from the cis face and progressed to the trans face. The reciprocal cis to trans shift occurred rapidly. It was observed after 5 min and was nearly complete by 15 min. Assuming that TGN membranes are Golgi apparatus-derived (Geuze and Morré 1991), it would follow that rapid membrane transfer and maturation had taken place upon transfer to 37 °C. Neither the diameter nor the number of cisternae per stack was diminished over the same time period as the accumulations of translocated membranes appeared. Thus, an amount of membrane equivalent to approximately 2 complete Golgi apparatus cisternae was observed to progress cis to trans within 15 min at 37 °C following the alleviation of the 16 °C block.

A low temperature block of the type reported here is expected to prove useful with the CRFK cells to aid in the study of viral glycoprotein processing. It is reasonable to expect that entry into the Golgi apparatus of endoplasmic reticulum-derived viral glycoproteins will be blocked at 16 °C, whereas transfer to 18 °C will then permit passage to the trans Golgi network but not beyond. Eventually, transfer to 24 °C or higher would permit exit from the trans Golgi network and delivery to the cell surface. In this manner, the studies reported here provide the basis for step-wise temperature control in CRFK cells of the passage of viral glycoproteins through the Golgi apparatus to permit investigation of the subcellular location and order of processing events in FIV maturation and assembly.

Acknowledgements

Electron microscope and darkroom facilities were provided through the courtesy of Dr. Charles Bracker. The excellent technical assistance of Mrs. D. A. Werderitsh is gratefully acknowledged. Work supported by a grant from the National Institutes of Health GM44675.

References

Brand M, Jansen E, Ploegh HL (1985) Effect of reduced temperature on glycoprotein (Ig. HLA) processing and transport in lymphoid cells. Mol Immunol 22: 787–794

Copeland CS, Zimmer KP, Wagner KR, Healey GA, Mellman I, Helenius A (1988) Folding, trimerization and transport are sequential events in the biogenesis of influenza virus hemagglutinin. Cell 53: 197–209

Fries E, Lindstrom I (1986) The effects of low temperatures on intracellular transport of newly synthesized albumin and haptoglobin in rat hepatocytes. Biochem J 237: 33–39

Geuze JM, Morré DJ (1991) The trans Golgi reticulum. J Electron Microsc Tech 17: 24–34

Griffiths G, Pfeiffer S, Simons K, Matlin K (1985) Exit of newly synthesized membrane proteins from the trans-cisternae of the Golgi complex to the plasma membrane. J Cell Biol 101: 949–964

Holmes KV, Doller EW, Sturman LS (1981) Tunicamycin resistant glycosylation of a coronavirus glycoprotein: determination of a novel type of viral glycoprotein. Virology 115: 334–344

Lagunoff D, Wan H (1974) Temperature dependence of mast cell histamine secretion. J Cell Biol 61: 809–811

Luft JM (1961) Improvements in epoxy resin embedding methods. J Biophys Biochem Cytol 9: 409–414

Matlin KS, Simons K (1983) Reduced temperature prevents transfer of a membrane glycoprotein to the cell surface but does not prevent terminal glycosylation. Cell 34: 233–243

Morré DJ, Paulik M, Nowack D (1986) Transition vesicle formation in vitro. Protoplasma 132: 110–113

– Minnifield N, Paulik M (1989) Identification of the 16 °C compartment of the endoplasmic reticulum in rat liver and cultured hamster kidney cells. Biol Cell 67: 51–60

Noguchi T, Morré DJ (1991) Vesicular membrane transfer between endoplasmic reticulum and the Golgi apparatus of a green alga, *Micrasterias americana*. A 16 °C block and reconstitution in a cell-free system. Protoplasma 162: 128–139

Nowack DD, Morré DM, Paulik M, Keenan T, Morré DJ (1987) Intracellular membrane flow: reconstitution of transition vesicle formation and function in a cell-free system. Proc Natl Acad Sci USA 84: 6098–6102

Paulik M, Nowack DD, Morré DJ (1988) Isolation of a vesicular intermediate in the cell-free transfer of membrane from transitional elements of the endoplasmic reticulum to Golgi apparatus cisternae of rat liver. J Biol Chem 263: 17738–17748

Phillips TR, Talbott RL, Lamont C, Muir S, Lovelace K, Elder JH (1990) Comparison of two host cell range variants of feline immunodeficiency virus. J Virol 64: 4605–4613

Saraste J, Kuismanen E (1984) Pre- and post-Golgi vacuoles operate in the transport of Semliki Forest virus membrane glycoproteins to the cell surface. Cell 38: 535–549

– Palade GE, Farquhar MG (1986) Temperature sensitive steps in the transport of secretory proteins through the Golgi complex in exocrine pancreatic cells. Proc Natl Acad Sci USA 83: 6425–6429

Talbott RL, Sparger EE, Lovelace KM, Filch WM, Pedersen NC, Lucico PA, Elder JH (1989) Nucleotide sequence and genomic organization of feline immunodeficiency virus. Proc Natl Acad Sci USA 86: 5743–5747

Tartakoff AM (1986) Temperature and energy dependence of secretory protein transport in the exocrine pancreas. EMBO J 5: 1477–1482

Tooze J, Tooze SA, Warren G (1984) Replication of coronavirus MHV-A59 in sac(–) cells: determination of the first site of budding of progeny virions. Eur J Cell Biol 33: 291–293

Tooze SA, Tooze J, Warren G (1988) Site of addition of N-acetyl-galactosamine to the E 1 glycoprotein of mouse hepatitis virus-A 59. J Cell Biol 106: 1475–1487