# Transport of Protein between Cytoplasmic Membranes of Fused Cells: Correspondence to Processes Reconstituted in a Cell-free System

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ABSTRACT   Mixed monolayers containing vesicular stomatitis virus-infected Chinese hamster ovary clone 15B cells (lacking UDP-N-acetylglucosamine transferase I, a Golgi enzyme) and uninfected wild-type Chinese hamster ovary cells were formed. Extensive cell fusion occurs after the monolayer is exposed to a pH of 5.0. The vesicular stomatitis virus encoded membrane glycoprotein (G protein) resident in the rough endoplasmic reticulum (labeled with [$^{35}$S]methionine) or Golgi complex (labeled with [$^3$H]palmitate) of 15B cells at the time of fusion can reach Golgi complexes from wild-type cells after fusion; G protein present in the plasma membrane cannot. Transfer to wild-type Golgi complexes is monitored by the conversion of G protein to an endoglycosidase H-resistant form upon arrival, and also demonstrated by immunofluorescence microscopy. G protein in the Golgi complex of the 15B cells at the time of fusion exhibits properties *vis à vis* its transfer to an exogenous Golgi population identical to those found earlier in a cell-free system (Fries, E., and J. E. Rothman. 1981. *J. Cell Biol.*, 90: 697–704). Specifically, pulse-chase experiments using the in vivo fusion and in vitro assays reveal the same two populations of G protein in the Golgi complex. The first population, consisting of G protein molecules that have just received their fatty acid, can transfer to a second Golgi population in vivo and in vitro. The second population, entered by G protein ~5 min after its acylation, is unavailable for this transfer, in vivo and in vitro. Presumably, this second population consists of those G-protein molecules that had already been transferred between compartments within the 15B Golgi population, in an equivalent process before cell fusion or homogenization for in vitro assays. Evidently, the same compartment boundary in the Golgi complex is detected by these two measurements. The surprisingly facile process of glycoprotein transit between Golgi stacks that occurs in vivo may therefore be retained in vitro, providing a basis for the cell-free system.

Reconstitution of intercompartmental protein transport is needed to permit the identification and study of the molecules that facilitate these processes and control their specificity. We have described a cell-free system in which a transport-coupled glycosylation is used to monitor movement of a glycoprotein between two Golgi membrane populations (1, 4, 6, 7, 22). One population of Golgi elements (from a mutant cell) contains the glycoprotein to be transported but lacks the needed glycosyltransferase. The other population of Golgi complex is from a wild-type cell that has the glycosyltransferase but not the glycoprotein substrate. The simplest view (20) is that this assay measures transfers between Golgi subcompartments, the idea being that such transfers can occur between subcompartments in different stacks and between those in the same stack. For example, one mechanism would be the budding or escape of a transport vesicle from one set of Golgi membranes followed by its fusion with another. Such an interpretation makes a major assumption, because *inter*-Golgi transfers have never been described in vivo. Indeed, their detection would normally be precluded by the simple fact that all of the Golgi stacks in a single cell function identically. A strong prediction from our studies with the cell-free system, however, is that inter-Golgi movement should occur in vivo, at least to some extent.

To enable such inter-Golgi transfers to be observed in whole cells, we have constructed hybrid cytoplasms in which two

distinguishable Golgi populations are present, by fusing mutant with wild-type cells. In this and the following article we report the occurrence of remarkably facile intercompartmental transfers between the two cytoplasms, including transfers between Golgi stacks. We show here that such transfers are apparently retained in and form the basis of the cell-free system. The accompanying article exploits the properties of this inter-Golgi transport to study the compartmental organization of the Golgi complex and to characterize the mechanism of protein transport within its stack.

We have employed the same strategy and cell types used in the reconstitution approach (1, 7, 20), the difference being that, for the cell-free system, membrane fractions isolated from the two cells were mixed and incubated, whereas for present purposes the cells themselves were fused. The cell population providing the transported glycoprotein is a Chinese hamster ovary (CHO)[1] cell mutant (clone 15B; references 8 and 25) that lacks a specific Golgi glycosyltransferase (N-acetylglucosamine [GlcNAc] transferase I) needed to initiate the steps resulting in the addition of peripheral GlcNAc, galactose (Gal), and sialic acid to form complex-type asparginyl (Asn)-linked oligosaccharides in the Golgi (10). Intracellular transport per se does not appear to be affected in this mutant (8, 25), so glycoproteins are transported to their correct locations but in incompletely glycosylated forms. The 15B cells are infected with vesicular stomatitis virus (VSV) so that they harbor the VSV-encoded membrane glycoprotein (G protein), whose transport is to be studied. Because of the GlcNAc transferase deficiency in clone 15B, the Asn-linked oligosaccharides of G protein are in high-mannose precursor forms that can be cleaved by endoglycosidase (Endo H) at all stages (rough endoplasmic reticulum [RER], Golgi, and plasma membrane) of its intracellular transport (2).

The cell population that receives and glycosylates the transported G protein is the wild-type CHO parent of clone 15B, whose Golgi complex contains an active GlcNAc transferase I. When the two types of cells are fused, G protein can potentially move from organelles in the infected (15B mutant) cells to the Golgi of the uninfected (wild-type) cells. When this occurs, G protein's incomplete oligosaccharide chains can be further glycosylated to yield mature, complex-type structures (10). This can be assayed directly by following incorporation of GlcNAc (or other sugars) into G protein, or indirectly by the conversion of G from an Endo H-sensitive to an Endo H-resistant form.

Key to the success of this scheme is a nontoxic method for a controlled, rapid, and complete cell fusion. Recent work has revealed that surface glycoproteins of enveloped viruses (including VSV G protein), although inert at physiological pH, become powerful and nonspecific fusogens in the pH range of 5.0-5.5 (14, 27). This acid pH mimics the interior of the endosomal compartment in which these viruses normally uncoat (14). In particular, exposure of a monolayer of cells expressing VSV G protein on their surface to pH 5 for as little as 30 s was shown to result in an extensive fusion within a few minutes to yield a syncytium capable of normal metabo-

lism for hours (27). These properties permit fusion to be carried out in the course of pulse-chase experiments, and allow bulk biochemical methods to be used for analysis. In our experiments, the VSV-infected 15B cells, in addition to donating G protein for transfers after fusion, express G protein at their surface and thereby allow an acid-initiated fusion to neighboring wild-type cells in a mixed monolayer.

## MATERIALS AND METHODS

*Cells and Virus:* VSV (Indiana serotype, San Juan isolate) stock was grown in baby hamster kidney cells (11). The temperature-sensitive Glasgow 31 mutant (18) was obtained from Dr. John Lenard (College of Medicine and Dentistry of New Jersey-Rutgers Medical School, Piscataway, NJ), and its temperature sensitivity was confirmed before use by plaque assay at 40° vs. 32°C. CHO clone 15B (8) and the parent CHO line (referred to as "parent") used for its selection were kindly provided by Dr. Stuart Kornfeld (Washington University, St. Louis, MO). These were grown in monolayer using Eagle's minimum essential medium (αMEM) (Gibco Laboratories, Grand Island Biological Co., Grand Island, NY) containing 7.5% fetal calf serum, penicillin (500 U/ml), and streptomycin (0.1 mg/ml). A wild-type CHO line (referred to as "wild-type," of different history from the 15B parent) was provided by Dr. Harvey Lodish (Massachusetts Institute of Technology, Cambridge, MA) and grown in suspension in the same αMEM growth medium.

*Preparation of Mixed Monolayers of VSV-infected 15B cells and Wild-type Cells:* A set of 6-cm plates was seeded with $1.2 \times 10^6$ cells of clone 15B the day before the experiment. The next day (when the cells had almost doubled) plates were infected with VSV, using ~10 plaque-forming units/cell (but see below). Specifically, VSV was added in 1 ml/plate of αMEM (without serum) containing 5 μg/ml actinomycin D and also 15 mM HEPES-KOH (pH 7.4). After 45 min at 37°C (with continuous rocking to facilitate absorption), the VSV-containing medium was removed, and growth medium supplemented with 15 mM $NH_4Cl$ was added. 15 min later this medium was replaced with 4 ml of a freshly trypsinized suspension containing a total of 1.6 $\times 10^7$ parent CHO cells in growth medium plus 15 mM $NH_4Cl$. For some control experiments, identically prepared 15B cells were substituted for parent cells at this stage. The mixed monolayers were rinsed once with Tris-saline medium (containing per liter: 8 g of NaCl, 0.38 g of KCl, 0.1 g of $Na_2HPO_4$, 0.1 g of $MgCl_2$, 0.1 g of $CaCl_2$, and 3 g of Tris, and titrated to pH 7.4 with HCl) containing 15 mM $NH_4Cl$ before use at 3.5-4 h postinfection.

The parent cell suspension used to overlay the infected 15B cells was prepared as follows. Confluent 10-cm monolayers of parent cells (containing ~1 × $10^7$ cells each) were treated with 15 mM $NH_4Cl$ in growth medium for 30 min to 1 h. Then, to release cells with trypsin, the monolayers were rinsed with TD medium (same as Tris-saline medium except that $CaCl_2$ and $MgCl_2$ are omitted) containing 15 mM $NH_4Cl$, and then quickly rinsed with 0.5% trypsin in TD containing 15 mM $NH_4Cl$. After ~1 min at room temperature, cells were detached by shaking and suspended in growth medium containing 15 mM $NH_4Cl$ to yield 4 × $10^6$ parent cells/ml, and used immediately to overlay the infected 15B cells. For some experiments, the "wild-type" line of suspension CHO cells was used in place of the parent of clone 15B. Identical results were obtained. For this purpose, 15 mM $NH_4Cl$ was added to the suspension culture 30 min to 1 h before use, then the cells were pelleted and resuspended in growth medium plus 15 mM $NH_4Cl$ to yield 4 × $10^6$ cells/ml.

The amount of VSV used to infect the 15B cells was optimized for each preparation of virus. With too low a multiplicity of infection, fusion is inefficient. With too high a multiplicity, fusion is too efficient: the syncytium becomes very fragile (sloughing off the plate) and protein synthesis is greatly inhibited after fusion. To determine the optimal amount of virus, the extent of fusion and the rate of protein synthesis before and after fusion (measured as in Table I) are examined as a function of dose of virus. A multiplicity (typically ~10 plaque-forming units/15B cell) is chosen, at which fusion is extensive but protein synthesis is not affected.

*Labeling and Fusion of Mixed Monolayers: [35S]Methionine (Met):* At 3.5-4 h postinfection, monolayers were pulse-labeled for 5 min with 2 ml/plate of Met-free MEM[2] supplemented with 7% dialyzed fetal calf

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[1] *Abbreviations used in this paper:* Asn, asparaginyl; CHO, Chinese hamster ovary; Endo H, endoglycosidase H; Gal, galactose; GlcNAc, N-acetylglucosamine; G protein, glycoprotein; αMEM, Eagle's minimum essential medium; Met, methionine; PBS, phosphate-buffered saline; RCA, *Riccinus communis* agglutinin (ricin); RER, rough endoplasmic reticulum; VSV, vesicular stomatitis virus.

[2] The Met-free MEM with normal levels of bicarbonate contained (per liter): NaCl (6.8 g), KCl (0.4 g), $MgCl_2 \cdot 6 H_2O$ (0.2 g), $NaH_2PO_4 \cdot H_2O$ (1.5 g), $NaHCO_3$ (2.0 g), HEPES (4.76 g), glucose (1.0 g), as well as the MEM amino acids except Met (at 1/50th the concentration specified by Gibco Laboratories for their 50× MEM Amino Acid

Rate of Protein Synthesis before and after Fusion of Mixed
Monolayers

| Time | Rate of protein synthesis |
| --- | --- |
| Before fusion | [1]* |
| 10 min after fusion | 0.96 |
| 30 min after fusion | 0.86 |
| 60 min after fusion | 0.92 |

Mixed monolayers were pulse-labeled with [$^{35}$S]Met (12.5 $\mu$Ci/ml) for 5 min as described in Materials and Methods either without fusion (no pH 5 treatment) or 10, 30, or 60 min after fusion. To determine the amount of $^{35}$S-labeled protein synthesized during the pulse, we extracted monolayers right after the pulse with 0.5 ml of the NaCl-Tris-EDTA-Triton-cholate buffer, and the extract was clarified as in Materials and Methods. Then, 0.5 ml of 10 mM Tris-HCl (pH 6.8), 2% SDS, and 30 mM dithiothreitol was added and the sample was boiled. A sample of 0.2 ml was taken, 50 $\mu$l of 10 mg/ml bovine serum albumin was added, and the mixture was precipitated with 5% trichloroacetic acid in a final volume of 1 ml. The precipitate was collected on a glass fiber filter, washed, dried, and counted for $^{35}$S.
* A total of 284,000 cpm of [$^{35}$S]Met was incorporated into acid-precipitable material in this plate during the 5-min pulse before fusion. The other values shown are relative to this.

serum, 15 mM NH$_4$Cl, and 25 $\mu$Ci/ml of [$^{35}$S]Met (New England Nuclear, Boston, MA). This step was carried out in a water bath; all other steps were in a CO$_2$ incubator unless otherwise indicated.

After the 5-min pulse, a variable period of chase was carried out before fusion. Labeling medium was aspirated, 4 ml/plate of chase medium (growth medium supplemented with 2.5 mM unlabeled Met, 15 mM NH$_4$Cl, and 15 mM HEPES [pH 7.4]) was added, and the monolayer was incubated for the desired period of chase before fusion. When no chase was desired, the plate was quickly rinsed with chase medium and immediately fused.

To fuse the mixed monolayers, chase medium was aspirated and replaced with warm pH 5 medium[3] also containing 15 mM NH$_4$Cl. After 1 min on a 37°C water bath, pH 5 medium was aspirated and replaced with 4 ml of the aforementioned chase medium. The monolayer was harvested 1 h after fusion and G protein was analyzed. In some experiments, cycloheximide (100 $\mu$g/ml) was added to the chase medium; the same results were obtained with or without this drug. In other experiments, G protein present in budded virions rather than in cells was analyzed. For this purpose, the chase medium at the time of harvest was centrifuged for 1 h at 45,000 rpm in the SW50.1 rotor to pellet virions, and the pellet was then treated in the same fashion as cells for analysis of G protein.

Labeling and Fusion of Mixed Monolayers: [$^3$H]Palmitate: At 3.5-4 h postinfection, monolayers were pulse-labeled for 5 min with 2 ml/plate of serum-free low-bicarbonate MEM[4] also containing 15 mM NH$_4$Cl and 0.5 mCi/ml of [9,10-$^3$H]palmitic acid (New England Nuclear). After the pulse, labeling medium was aspirated and the monolayers were quickly rinsed with warm Tris-saline medium containing 15 mM NH$_4$Cl and 1 mg/ml of defatted bovine serum albumin (Sigma Chemical Co., St. Louis, MO) to remove excess [$^3$H]palmitate. Then a variable period of chase before fusion was begun by adding 4 ml of the serum-free low-bicarbonate medium now containing 40 $\mu$M of unlabeled palmitic acid as well as 15 mM NH$_4$Cl. When no chase before fusion was desired, monolayers were quickly and successively rinsed with the defatted albumin and the palmitate-containing media, and fused immediately.

Solution), 1× nonessential amino acids (from the 100 × MEM Nonessential Amino Acid Solution by Gibco Laboratories), 1× MEM vitamins (from the 100× MEM Vitamin Solution by Gibco Laboratories), and phenol red (0.001%), adjusted to pH 7.4 with NaOH.
[3] The fusion medium was very similar to that used by White et al. (27). It contained (per liter): NaCl (8.1 g), KCl (0.2 g), Na$_2$HPO$_4$ (1.56 g), HEPES (2.38 g), morpholine ethane sulfonic acid (1.95 g), CaCl$_2$· 2H$_2$O (0.062 g), MgSO$_4$ (0.082 g), glucose (1.0 gm), bovine serum albumin (2.0 g), 1× MEM amino acids (from the 50× MEM Amino Acid Solution made by Gibco Laboratories), 1× MEM nonessential amino acids, 1× MEM Vitamin Solution, and phenol red (0.001%), titrated to pH 5.0 with NaOH.
[4] The low-bicarbonate MEM contained (per liter): NaCl (8.1 g), KCl (0.2 g), Na$_2$HPO$_4$ (1.56 g), NaHCO$_3$ (0.1 g), glucose (1.0 g), HEPES (7.14 g), 1× MEM nonessential amino acids, 1× MEM vitamins, and phenol red (0.001%), adjusted to pH 7.4 with NaOH.

To fuse the monolayers after the variable period of chase, we replaced the medium with 2 ml of a pH 5 medium (the same as used for the [$^{35}$S]Met experiments but also containing 100 $\mu$g/ml cycloheximide). After 1 min at 37°C, pH 5 medium was replaced with 4 ml of serum-free growth medium containing 40 $\mu$M palmitate, 100 $\mu$g/ml cycloheximide, 10 mM HEPES (pH 7.4), and 15 mM NH$_4$Cl. After 1 h of further incubation, the monolayers were harvested and G protein was analyzed. Cycloheximide improves the efficiency of the chase by eliminating the pool of nascent G protein into which fatty acid is incorporated. All of the steps except the last hour of incubation need to be performed in a 37°C water bath to facilitate the speed of the required operations. The use of the low-bicarbonate medium assures that a constant pH is maintained outside a CO$_2$ incubator.

Immune Precipitation and Analysis of G Protein from Fused Monolayers: Monolayers were rinsed with ice-cold Tris-saline buffer, and incubated with 0.5 ml of 0.1 M NaCl, 25 mM Tris-HCl, pH 7.5, 2.5 mM Na$_2$EDTA, 0.5% Triton X-100, 0.5% sodium cholate for ~1 min at 37°C to extract G protein from cells. The extract was clarified by centrifuging for 5 min at about 12,000 g in a microfuge, and the supernatant was used for immune precipitations. For $^{35}$S-labeled cell extracts, 100-$\mu$l aliquots were immuneprecipitated by incubation overnight at 4°C with 15 $\mu$l of a rabbit anti-G protein serum (prepared as described [1]), or preimmune serum as a control. Then, 25 $\mu$l of a 10% suspension of fixed Staphylococcus aureus cells (Calbiochem-Behring Corp., San Diego, CA) was added. After 1 h at 4°C, the cells were pelleted (15 s in the microfuge) and washed three times (1 ml per wash) with 0.2 M NaCl, 5 mM Na$_2$EDTA, 1% Triton X-100, 50 mM Tris-HCl (pH 7.5). The S. aureus cells were suspended in 80 $\mu$l of 50 mM Tris-HCl (pH 6.8), 1% SDS, 15 mM dithiothreitol, and boiled for 2 min to release G protein. S. aureus cells were centrifuged out (2 min in the microfuge) and a 60-$\mu$l aliquot of the supernatant was incubated with 40 $\mu$l of 0.01 IU/ml Endo H (dissolved in 0.3 M citric acid-NaOH buffer [pH 5.5] containing 0.1% SDS). Endo H was purchased from the Division of Laboratories and Research, New York State Department of Health, Albany, N.Y. Endo H was allowed to digest G protein overnight at 37°C. Then, G protein was precipitated with 0.3 ml of 10% trichloracetic acid and dissolved by boiling in sample buffer (neutralizing pH by adding 1 M Tris base) and electrophoresed in a 10% polyacrylamide gel according to Laemmli (12). The gel was treated with ENHANCE (New England Nuclear) for fluorography, dried, and autoradiographed. In a typical $^{35}$S experiment (such as Fig. 2), three 100-$\mu$l aliquots of cell extract were separately analyzed. Two were precipitated with anti-G serum, one with control serum. One of the anti-G protein precipitates and the control precipitate were digested with Endo H before electrophoresis as just described. The other anti-G protein precipitate was mock-digested by omitting Endo H from the overnight incubation with the citrate-SDS buffer.

For [$^3$H]palmitate-labeled cell extracts, the entire extract (0.5 ml) was immunoprecipitated with anti-G protein serum (75 $\mu$l) and collected on S. aureus cells (125 $\mu$l) as described for the smaller-scale incubation used for $^{35}$S-labeled extracts. Then, the [$^3$H]G protein was released by boiling in 130 $\mu$l of the Tris-SDS-dithiothreitol buffer, and an 120-$\mu$l sample of this fraction was incubated with 80 $\mu$l of 0.01 IU/ml Endo H, precipitated, electrophoresed, and autoradiographed as before.

Immunofluorescence Analysis of Mixed Monolayers: For these experiments, mixed monolayers were prepared as before except that a mutant of VSV (ts G31) with a temperature-sensitive M protein, defective in budding at 40°C, was used (18). Intracellular transport of G protein is not affected by this mutation (21). Infection was initiated at 32°C and 1 h later the temperature was shifted to 40°C and maintained at 40°C thereafter. For these experiments, wild-type cells were added at 2 h postinfection. Cycloheximide (100 $\mu$g/ml) was added 15 min before fusion to ensure that all G protein demonstrated by immunofluorescence had been synthesized in 15B cells before fusion. Fusion was initiated at 3.5 h postinfection by exposure of the monolayer to pH 5 medium (38.5°C) for 1 min. Then, the monolayer was returned to growth medium (plus cycloheximide and with NH$_4$Cl) at 38.5°C for 20 min, and the cells were fixed in a 2 → 8% paraformaldehyde gradient as described (3). In controls, the fusion step was omitted.

For fluorescence studies, randomly chosen areas of the mixed monolayer were immunoincubated to reveal the antigens to be localized. Areas of the plate were delineated for separate incubations by using vaseline-coated rubber rings of 0.3 cm inside diameter. We used plastic tissue culture plates for this purpose because the infected 15B cells did not adhere well to the glass coverslips generally used for immunofluorescence studies.

Some areas of the monolayer were immunoincubated to stain simultaneously for G protein and for Ricinus communis agglutinin—ricin (RCA)-binding sites in double-label immunofluorescence. Other areas of the same plate were incubated to stain for a 135-kilodalton (kD) Golgi-membrane protein (15) and intracellular RCA-binding sites. For simultaneous RCA and G-protein localizations, external RCA sites were first saturated by incubation with 50 $\mu$g/ml

RCA in phosphate-buffered saline (PBS) for 30 min. By so doing, RCA-fluorescein, applied later in the procedure after permeabilization, will be restricted to intracellular binding sites. Next, cells were permeabilized with saponin (0.05% in PBS), and then incubated with 50 μg/ml of monoclonal anti-G antibody (kindly provided by Dr. Douglas Lyles, Bowman Gray School of Medicine, Winston-Salem, NC; clone I1 [13]) in PBS overnight at 20°C followed by 50 μg/ml rabbit anti-mouse IgG (N. L. Cappel Laboratories Inc., Cochranville, PA), in PBS for 60 min, and then 50 μg/ml rhodamine-conjugated goat anti-rabbit IgG (Vector laboratories, Burlingame, CA) for 60 min in PBS. Finally, the areas were incubated with 50 μg/ml protein A (Pharmacia Fine Chemicals, Piscataway, NJ) for 30 min in PBS before incubating with 50 μg/ml fluorescein isothiocyate-RCA (Vector Laboratories) for 30 min. Between all steps the cells were extensively washed with PBS/glycine buffer (pH 7.4; 1.5 g glycine per liter of PBS). Similar results were obtained when the incubation orders were reversed. Controls carried out with nonimmune rabbit and mouse IgG showed no staining for G protein as did controls on uninfected cells. Fluorescein isothiocyanate-RCA binding was eliminated by a prior Gal (100 μg/ml) incubation. In most experiments unfused 15B cells serve as internal negative controls to validate the specificity of the RCA stain.

The same procedure was used for the simultaneous localization of RCA-binding sites and the 135 kD Golgi membrane protein by double immunofluorescence except in this case the rabbit anti-Golgi antibody (50 μg/ml) was used in place of the mouse monoclonal anti-G antibody. The intermediate treatment with rabbit anti-mouse was omitted.

## RESULTS

### Formation, Fusion, and Properties of Mixed Monolayers

Mixed monolayers composed of ~20% VSV-infected clone 15B cells and 80% uninfected wild-type CHO cells were prepared as follows. Clone 15B cells were grown on plates until ~20% confluent, and were infected with VSV (Fig. 1a). 45 min after the initiation of infection, VSV-containing medium was removed and replaced with medium containing 15 mM NH₄Cl. Ammonium chloride and other lysosomotropic amines prevent the initiation of new rounds of VSV infection but do not interfere with ongoing VSV replication (14). They do so by raising the pH of lysosomes and endosomes. At 1 h postinfection, medium was replaced with a suspension of freshly trypsinized wild-type CHO cells (usually the parent of the 15B mutant) in NH₄Cl-containing medium. These wild-type cells had previously been treated with NH₄Cl for 30 min to 1 h while in monolayer. By 3.5 h postinfection, the wild-type cells had attached to and spread out on the dish to form

a confluent mixed monolayer with the infected 15B cells (Fig. 1b). Excess wild-type cells are readily removed by rinsing the mixed monolayer just before the experiment.

Fusion of cells can be initiated by exposing the mixed monolayer to medium adjusted to pH 5.0 for 1 min at 37°C (27). Upon return to physiological medium (pH 7.4), fusion among neighboring cells begins within ~1 min at 37°C and by 10 min fusion is complete yielding very large multicellular syncytia (Fig. 1c). This treatment and the resulting fusion do not affect the processes under study. Protein synthesis occurs at the same rate before and after fusion (Table I) as first reported by Helenius and co-workers (27). Also, the rate of protein transport between compartments in the Golgi complex is the same before, during, and after fusion. This quantity was measured as the time taken for Gal to be added to G protein after GlcNAc addition in the Golgi complex (Fig. 4 in the following article [23]).

Ammonium chloride is used to prevent infection of wild-type cells by progeny VSV budded from infected 15B cells; in so doing, it will block the intracellular uncoating but not the endocytosis of viral particles (14, 17). As expected, the wild-type cells do endocytose VSV, as G protein antigen is found in their cytoplasm by immunofluorescence (data not shown). However, these wild-type cells are not infected with VSV because all of the G protein synthesized by the unfused mixed monolayer remains Endo H-sensitive (Fig. 2, set 1) and is therefore synthesized entirely in 15B cells. Any G protein synthesized in wild-type cells would have been Endo H-resistant after the 1-h chase in this experiment. When 15 mM NH₄Cl was added to cells together with VSV rather than 45 min after VSV, infection was prevented as reported (17). This was shown by the lack of transcription of viral RNA (data not shown), measured according to Miller and Lenard (17).

A possible concern was whether the inclusion of NH₄Cl would affect the transport processes under study. However, data in Fig. 5 of the following article (23) show that 15 mM NH₄Cl has no effect upon (a) the rate of G protein synthesis, (b) the overall rate and efficiency of transport of G protein from RER to budded viral particles, or (c) the rate and efficiency of intercompartmental transport in the Golgi complex.
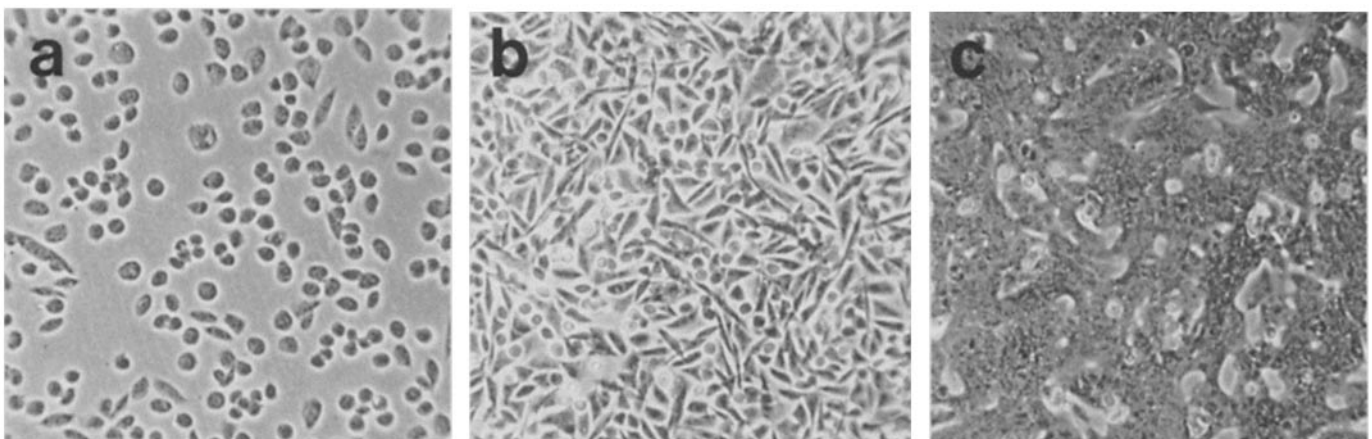


FIGURE 1  Stages in the preparation and use of a mixed monolayer. (a) VSV-infected clone 15B cells 30 min after infection, before wild-type cells are added. Note that these are rounded up due to the VSV infection. (b) Confluent mixed monolayer at 3.5 h after infection, now containing uninfected wild-type cells which spread out and fill all of the available surface. (c) Mixed monolayer 10 min after a 1-min exposure to a pH of 5. Nearly quantitative fusion has yielded a syncytium. Monolayers were fixed with methanol, stained with Giemsa. Phase-contrast micrographs.
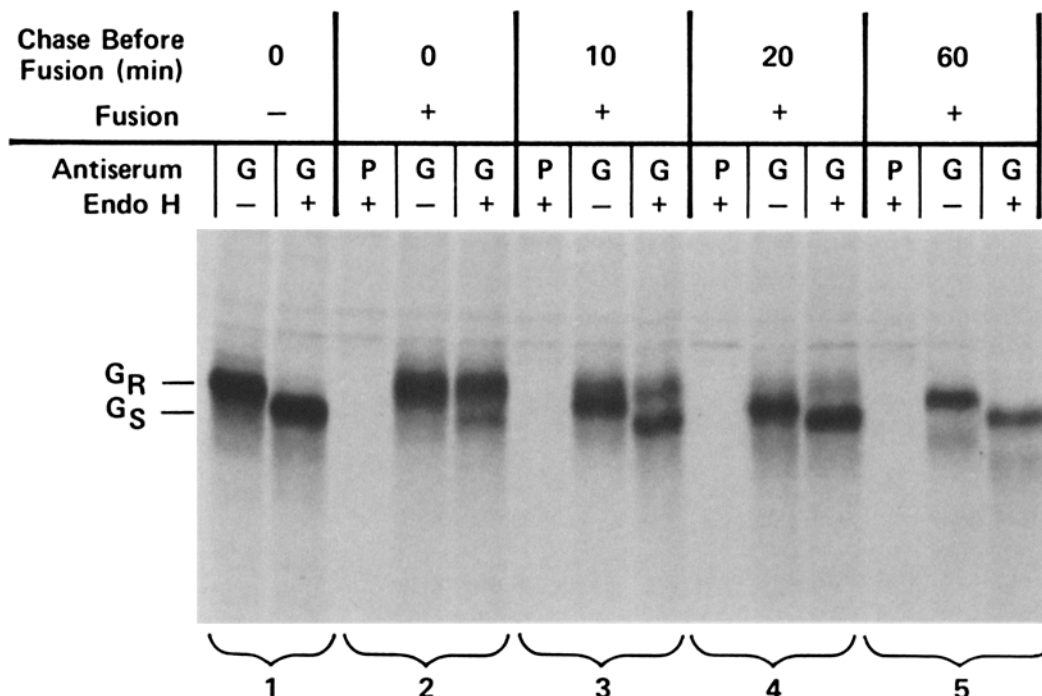
| Chase Before Fusion (min) | 0 | | 0 | | | 10 | | | 20 | | | 60 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Fusion | − | | + | | | + | | | + | | | + | | |
| Antiserum | G | G | P | G | G | P | G | G | P | G | G | P | G | G |
| Endo H | − | + | + | − | + | + | − | + | + | − | + | + | − | + |

$G_R$ —
$G_S$ —

  1      2      3      4      5

FIGURE 2 Conversion of G protein to Endo H resistance after fusion of mixed monolayers. Monolayers were pulse-labeled with [$^{35}$S]Met and chased for 0, 10, 20, or 60 min before fusion. 1 h after fusion G protein was immunoprecipitated from cells, digested with Endo H, electrophoresed in an SDS gel, and autoradiographed for 3 d. Shown here is a portion of the autoradiograph containing the G-protein bands. Controls included the use of preimmune rather than anti-G serum (indicated by "P" rather than by "G"), a mock-digestion in which Endo H was omitted (indicated by a minus) and omission of the pH 5 treatment so as to eliminate fusion (indicated by a minus). Group 1: fusion was omitted, cells were harvested after 1-h chase. Groups 2–5: fused after 0, 10, 20, and 60 min of chase after the [$^{35}$S]Met pulse. $G_S$, Endo H-sensitive form of G protein resulting from cleavage of oligosaccharides; $G_R$, Endo H-resistant form of G protein whose oligosaccharides cannot be cleaved by Endo H.

## Transfer of G Protein from RER but Not from Plasma Membrane after Cell Fusion

Mixed monolayers were pulse-labeled for 5 min with [$^{35}$S]Met to label G protein in the RER of the 15B cells, chased for various periods to allow G to be transported along the RER–Golgi complex–plasma membrane pathway, and then fused to permit transfer of $^{35}$S-labeled G protein to compartments in neighboring wild-type cells. Transfer to the Golgi complex from wild-type cells was monitored by the conversion of $^{35}$S-labeled G from Endo H-sensitive to Endo H-resistant forms. A period of 1 h after fusion was allowed for this transport and glycosylation to occur, at which time monolayers were harvested and extracted with detergent, and G protein was immunoprecipitated. The immunoprecipitate was then treated with Endo H or mock-digested, and electrophoresed in an SDS gel and autoradiographed. The gel from a typical experiment is shown in Fig. 2, and the results of two independent fusion experiments are quantitated in Fig. 3. The same results were obtained when G protein in VSV particles budded into the medium, rather than G protein in cells, was analyzed (data not shown). All of the $^{35}$S-labeled G protein in these experiments must have been synthesized in the VSV-infected 15B cells before fusion, in that a chase was begun before the pH 5 treatment (and in some cases cycloheximide was added at the time of chase, as in Fig. 3).

When the cells are fused right after the pulse, at a time when almost all of G still resides in RER, ~70% of the $^{35}$S-labeled G protein is eventually converted to an Endo H-resistant form. This suggests that G protein residing in the
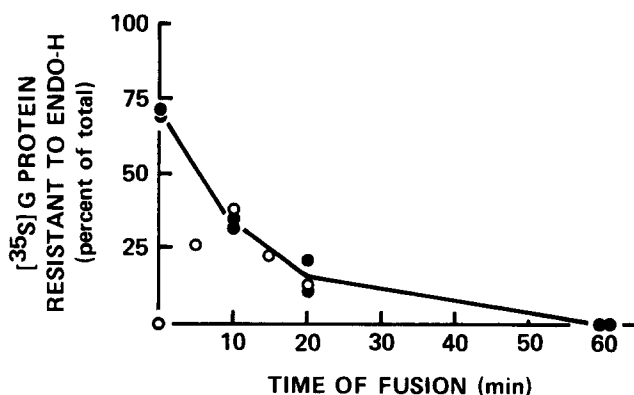


FIGURE 3 Percent of $^{35}$S-labeled G protein rendered Endo H resistant as a result of cell fusion, as a function of the time of chase at which fusion was initiated. Quantitated from densitometer tracings of autoradiographs. (●) Data from Fig. 2 and a second experiment representing the results for fusion of mixed monolayers. In the second experiment, cycloheximide (100 μg/ml) was present during the 1-h chase period after fusion. (○) Data of Fries and Rothman (7) replotted for comparison, representing the results of incubation of membranes from [$^{35}$S]Met-labeled VSV-infected 15B cells (prepared at the indicated times of chase) with membranes from wild-type cells.

RER of the 15B cells at the time of fusion can be transferred to reach the Golgi complex eventually from the wild-type cells. As mentioned earlier, no processing to Endo H-resistance is observed when the pH 5 step (i.e., fusion) is omitted (Fig. 1, set 1).
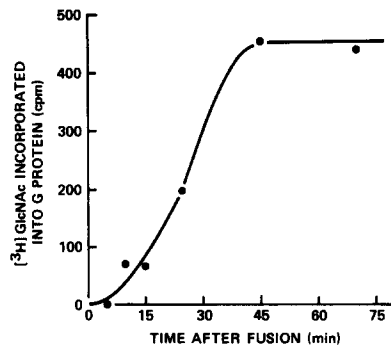
FIGURE 4  Kinetics of incorporation of GlcNAc into G protein after fusion. Mixed monolayers were first treated for 5 min with growth medium containing 100 μg/ml cycloheximide, 15 mM NH₄Cl, and 15 mM HEPES pH 7.5, and then fused via an incubation of 1 min at 37°C with pH 5 medium also containing NH₄Cl and cycloheximide. The pH 5 medium was immediately aspirated and replaced with 1.5 ml of a low-glucose medium containing 67 μCi/ml of [6-³H]glucosamine (New England Nuclear). This consisted of the Met-free MEM² with 0.1 g/liter glucose in place of 1 g/liter glucose, supplemented with 0.25 mM Met, and also contained 7% dialyzed fetal calf serum, 100 μg/ml cycloheximide, and 15 mM NH₄Cl. At indicated times after fusion, monolayers were rinsed, and extracted with detergent and immunoprecipitated as described in Materials and Methods. For each plate, a 200-μl aliquot of the cell extract was precipitated with anti-G serum and another 200-μl aliquot was precipitated with preimmune serum. The [³H]GlcNAc-labeled G protein was released from S. aureus cells by boiling in SDS buffer as described in Materials and Methods, and counted. Plotted is the amount of ³H incorporated into G protein (the difference between the radioactivity in the anti-G precipitate and the preimmune control) versus the time after fusion at which the monolayer was harvested.

When a period of intracellular transport is allowed before fusion, the fraction of ³⁵S-labeled G protein converted to Endo H-resistance upon fusion diminishes, by a factor of ~2 for every 10 min of chase. By 1 h, at which time most of the ³⁵S-labeled pool of G protein is present in the plasma membrane of the infected cells (11, 21), G protein is no longer detectably converted to Endo H-resistance. This suggests that G protein residing in the plasma membrane of 15B cells at the time of fusion cannot be transferred to the Golgi complex from wild-type cells.

Fig. 4 shows that transfers to the wild-type Golgi complex are completed within 45 min after fusion. Here the incorporation of [³H]GlcNAc into G protein in the wild-type Golgi complex was measured directly. For this purpose, mixed monolayers were fused and then [³H]glucosamine was added to the medium; G protein was immunoprecipitated from plates harvested at various times after fusion. Cycloheximide was added just before fusion and maintained throughout, serving two connected purposes: (a) to ensure that only transfers of G protein made in 15B cells before fusion will be measured; and (b) to stop the co-translational incorporation of GlcNAc into the oligosaccharide core of G protein in the RER. This ensures that [³H]GlcNAc will be incorporated into G protein only in the Golgi complex of the wild-type cells and not in the RER. No significant incorporation into the immunoprecipitate was found in the following control monolayers in which (a) 15B cells were mock-infected, (b) wild-type cells were replaced with uninfected 15B cells, and (c) the fusion step was omitted (data not shown).

One trivial explanation of all of these results would be that

GlcNAc transferase I activity, normally missing from 15B Golgi membranes, is somehow activated or corrected rapidly upon fusion with wild-type cells; this would enable the observed glycosylation of G to occur in situ in the 15B membranes without need for transfer to the wild-type Golgi complex. If this were the case, the total activity of transferase I in mixed monolayers should increase after fusion. To test this, membranes were prepared from wild-type cells, 15B cells, and from mixed monolayers containing ~20% wild-type cells and 80% 15B cells that had or had not been fused. The specific activity of GlcNAc transferase I was found to be the same for the fused and unfused mixed monolayers (Table II), in between the value for 15B and parent cells. Galactosyltransferase, another Golgi glycosyltransferase, served as an internal control and had the same specific activity in all four membrane preparations.

## Transfer of Freshly Acylated G Protein

A significant fraction (~35%) of the ³⁵S-labeled G protein in 15B cells can still be transferred to wild-type Golgi complex when fusion is carried out after a 10-min chase (Fig. 3). G protein is mostly within the Golgi of the 15B cells at this time of chase (2, 4, 7), raising the possibility that G protein can be transferred between the two Golgi populations after fusion.

To examine this possibility more closely, it is necessary to label the G protein in the Golgi complex of 15B cells selectively before fusion, and then see if that population of G is rendered Endo H-resistant after fusion to wild-type cells. Fatty acid is added covalently to G at ~10 min after polypeptide chain synthesis, either in the Golgi complex or moments before G enters the Golgi complex, as judged by morphological, kinetic, and cell fractionation studies (2, 4, 19, 24). In particular, we have shown that the majority of G protein pulse-labeled with [³H]palmitate in VSV-infected 15B cells co-fractionates with Golgi membranes on sucrose gradients and has trimmed (mannose₅) oligosaccharide chains (4).

TABLE II

Lack of Effect of Fusion on Glycosyltransferase Activities

| | | Specific activity | |
| | | Gal trans-ferase | GlcNAc transfer-ase I |
| Composition of monolayer | Fusion | (cpm/μg) | (cpm/μg) |
| A. All 15B cells | No | 17 | 0.2 |
| B. All parent cells | No | 20 | 52 |
| C. 20% parent and 80% 15B | No | 16 | 16 |
| D. 20% parent and 80% 15B | Yes | 22 | 16 |

Membranes were prepared from mixed monolayers of different compositions that had or had not been fused, and assayed for activities of galactosyltransferase and GlcNAc transferase I. Each of the four membrane preparations made (corresponding to the four lines of data in the Table) is derived from 10 identical confluent 10-cm plates of cells. For A, confluent 15B cell monolayers were used. For B, confluent parent cell monolayers were used. For C and D, monolayers were seeded with a mixture of 20% parent and 80% 15B cells and grown to confluency. All plates were infected with VSV at a multiplicity of 5; so, both 15B and parent cells were infected in this experiment. At 2.5 h after infection cells for preparation D were fused by a 1-min treatment at pH 5. Preparations A–C were not fused. After 1 h of incubation in growth medium, cells were scraped, homogenized, and the postnuclear supernatant prepared as described (4, 5). Total membranes were pelleted from this supernatant (SW 50.1 rotor, 40,000 rpm for 30 min) and resuspended in 1 ml of 1 mM Tris–HCl, pH 8.0, for assay. Galactosyltransferase and GlcNAc transferase I were assayed (5). Protein concentration was measured using the Lowry method (16). Shown are the specific activities (counts per minute of [³H]Gal or [³H]GlcNAc transferred from the UDP-sugar to glycoprotein acceptor per microgram of membrane added).
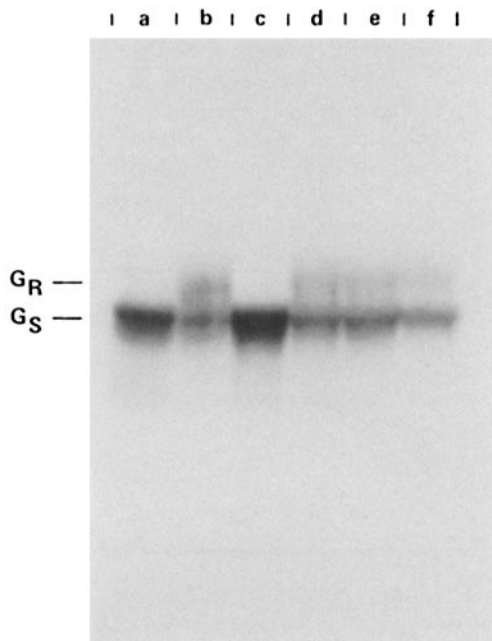
FIGURE 5 Conversion of [³H]palmitate-labeled G protein to Endo H-resistance after fusion of mixed monolayers. Monolayers were pulse-labeled with [³H]palmitate and chased for 0, 5, 10, or 30 min before cell fusion. 1 h after fusion G protein was immunoprecipitated from cells, digested with Endo H, electrophoresed on an SDS gel, and autoradiographed for 2 wk. Shown here is the portion of the autoradiograph containing the G protein bands. Controls include not fusing the monolayer, and fusing the infected 15B cells with uninfected 15B cells rather than wild-type cells. (Lane a) The fusion step (pH 5 treatment) was omitted and cells were harvested after 1-h chase. (Lanes b-f) Fused after 0, 0, 5, 10, and 30 min of chase, respectively. The plate used for lane c was the control in which uninfected 15B cells replaced uninfected wild-type cells in the preparation of the mixed monolayer. $G_R$ and $G_S$ are defined as in the legend to Fig. 2.

Therefore, G protein freshly labeled with [³H]palmitate almost certainly resides in the Golgi of 15B cells at the time of fusion.

Fig. 5 shows the autoradiograph of a fusion experiment in which mixed monolayers were pulse-labeled with [³H]palmitate, and Fig. 6 presents quantitative data from this and two additional experiments. When the cells were fused right after the pulse of [³H]palmitate, 40–45% of the ³H-G protein was subsequently made Endo H-resistant (Fig. 5, lane b), presumably after transport to Golgi complex derived from wild-type cells. No G was made Endo H-resistant when the fusion step was omitted from the protocol (Fig. 5, lane a), as expected. An additional control was to replace the wild-type cell population with uninfected clone 15B cells; as expected, no Endo H-resistant G protein was formed after fusion (Fig. 5, lane c).

When a chase is allowed to permit additional transport of [³H-palmityl]-G protein to occur before fusion with wild-type cells, the fraction of G protein that will eventually pass into exogenous wild-type Golgi complex (to receive GlcNAc and become Endo H-resistant) declines precipitously, with a half time of ~5 min. This is the same time course with which freshly acylated G is converted to Endo H resistance in the Golgi of wild-type CHO cells (4).

These data suggest that G protein residing in one Golgi population (in 15B cells) at the time of fusion can transfer to another Golgi population (from wild-type cells) after fusion.

They also distinguish in this regard two populations or pools of G protein that exist after fatty acylation. The first pool, consisting of G protein molecules that have just received their fatty acid, will transfer to a second Golgi population. The second pool, entered by G protein ~5 min after its acylation, is unavailable for this transfer; presumably these G protein molecules had already been transferred within the 15B cell Golgi population in an equivalent process before cell fusion. Therefore, it is likely that both pools reside in the Golgi complex and consist of distinct compartments. This behavior is the focus of the accompanying paper, and the correctness of this interpretation is established by the evidence presented therein.

## Comparison of Results Obtained by Cell Fusion with Those from a Cell-free System

We have reported analogous experiments using a cell-free system in which a crude ("donor") membrane fraction from VSV-infected 15B cells was incubated with a comparable ("acceptor") membrane fraction from uninfected wild-type cells (4, 7). Are the same populations of G protein molecules that are subject to transfer in vivo also transferred in vitro?

G protein originating in 15B cell membranes was rendered Endo H-resistant in incubations with wild-type CHO membranes provided that the high-speed supernatant fraction, ATP, and UDP–GlcNAc were also added. A pulse-chase protocol similar to that used in the in vivo fusion studies was carried out in which the total membrane fraction was prepared from VSV-infected 15B cells at various times of chase after pulses of [³⁵S]Met (7) or [³H]palmitate (4), and tested to see which populations of G protein in the cell were available for the transport-coupled glycosylation in vitro.

The results of these studies are replotted as the open circles in Figs. 3 and 6 for purposes of comparison with the present cell fusion results. Whereas most of the G protein pulse-labeled with [³⁵S]Met (residing in the ER of 15B cells at the time of fusion) was glycosylated (to achieve Endo H resist-
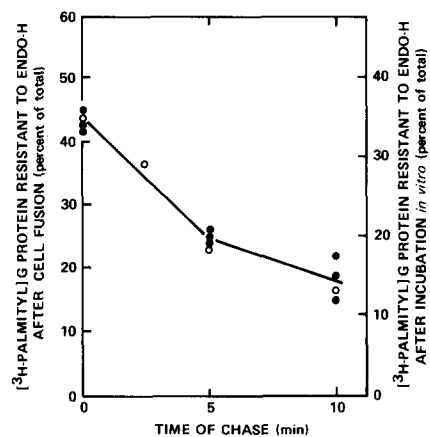


FIGURE 6 Percent of [³H]palmitate-labeled G protein rendered Endo H-resistant as a result of cell fusion, as a function of the time of chase at which fusion was initiated. Determined from densitometer tracings of autoradiographs. (●) Data from Fig. 5 and two additional experiments representing the results for fusion of mixed monolayers. (○) Data of Dunphy et al. (4) replotted for comparison, representing the results of incubation of membranes from [³H]-palmitate-labeled VSV-infected 15B cells (prepared at the indicated time of chase) with membranes from wild-type cells. Note that the vertical scales have been adjusted so as to equate the values at 0-min chase for the two types of experiments.
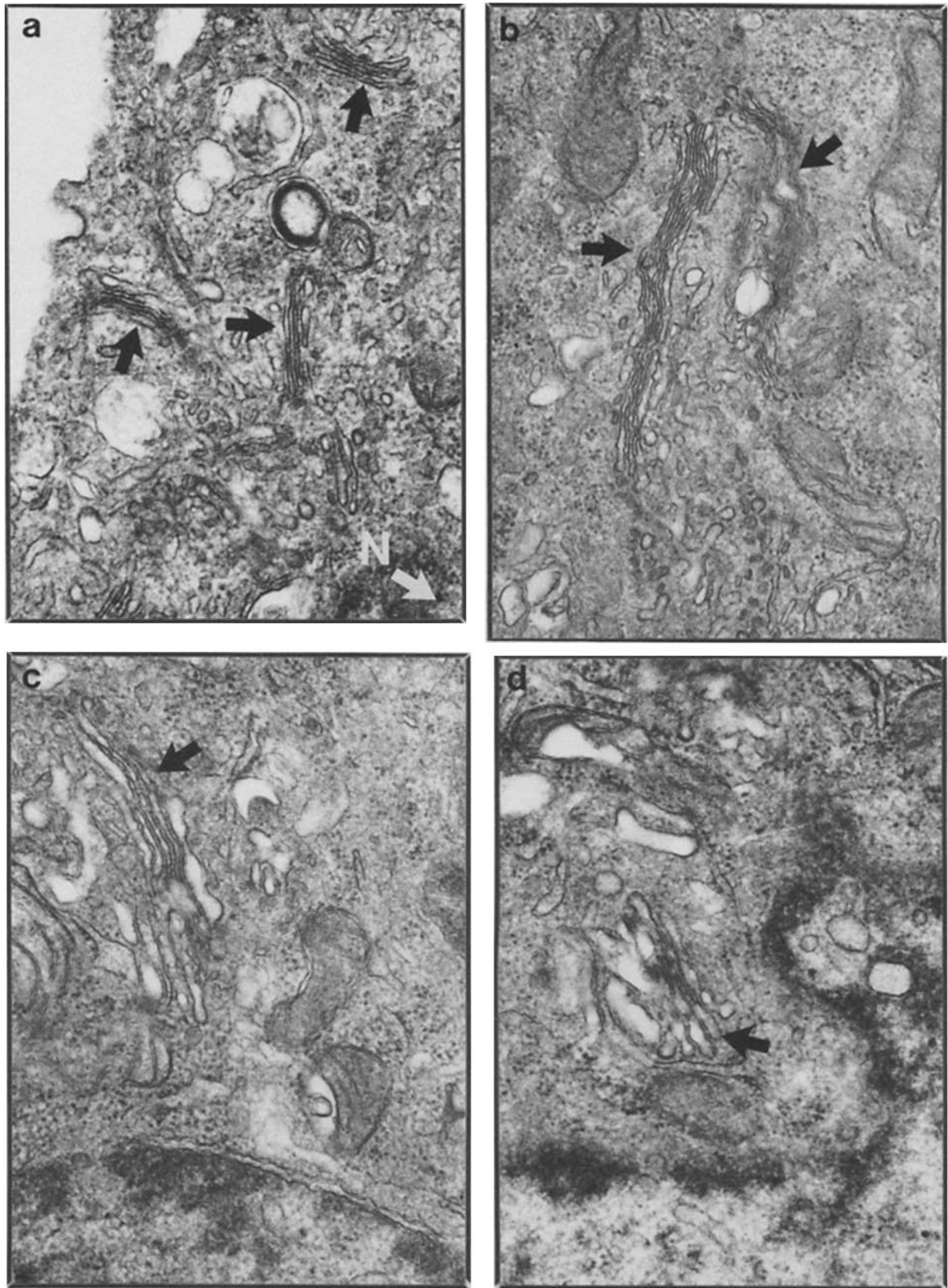
FIGURE 7  Morphology of Golgi stacks before and after cell fusion. (a) Before fusion; (b–d) after fusion. The nucleus (N) is at the lower right corner of a, just outside this photograph. Portions of nucleus are shown in b and d. About × 40,000.

ance) after fusion, none was available when membranes were prepared and incubated in the cell-free system (O). As the labeled population of G protein is being transported (during the chase period) out of the RER towards the Golgi complex, much more of G protein becomes available in vitro (O) and less in vivo (●) until the 10-min point is reached, at which

point most of G is in the Golgi membranes (2, 4, 7). From that point on, there is a striking quantitative agreement between these two very different types of experiments.

This parallel, commencing about when G protein enters the Golgi complex, is especially evident when [³H]palmitate is used to label the G-protein pool selectively that has just arrived in the Golgi complex (Fig. 6). The extent of transfer to the second Golgi population is very similar in vivo (●) and in vitro (○); moreover, the effect of further transport (chase) is the same (note that the vertical scales for the two sets of data in Fig. 6 are different and have been adjusted to normalize the initial values so as to emphasize the ensuing time course).

Three conclusions follow from this comparison: (a) G protein contained in RER can be transferred to exogenous Golgi in vivo but not, under present conditions, in vitro; (b) the same population of freshly acylated G protein molecules that can be transferred to exogenous Golgi complexes after fusion in vivo can also be glycosylated by exogenous Golgi complexes when membranes are incubated in vitro; (c) the quantitative similarity of the extent of transport in vivo and in vitro also implies that the G protein population in the pertinent Golgi compartment is mostly or completely trans-

ported to wild-type Golgi complexes in the cell-free system.

It is striking that the same two populations of G protein are distinguished using such very different kinds of assays. The underlying significance of these two pools in terms of compartmental subdivisions of the Golgi complex is discussed in the following paper.

## Visualization of the Transfer of G Protein after Cell Fusion

A priori there is no reason to suppose that the two Golgi populations fuse after cell fusion, in that the pH 5 treatment only pertains to the exterior of the cell. Indeed, examination of cells before and after fusion does not reveal qualitative changes in the morphology or distribution of Golgi stacks at the electron microscope level (Fig. 7).

We have employed immunofluorescence microscopy to directly demonstrate that the Golgi stacks from 15B and wild-type cells remain distinct following fusion of mixed monolayers, and to demonstrate the appearance of G protein in wild-type Golgi regions. In a double-label experiment (Fig. 8), RCA was used to stain Golgi complexes (9, 26) from wild-



**Ricin (FITC)**  **Anti-Golgi (Rhod)**

FIGURE 8  Double immunofluorescence showing that Golgi complexes derived from wild-type and 15B cells remain distinct after cell fusion. Double-labeling experiment for RCA-binding sites (a and c) and Golgi membrane protein (b and d) before (a and b) and after (c and d) fusion. Distinct Golgi complexes can be discerned, which remain either RCA positive (arrows) or negative (arrowheads) after fusion. The RCA stain in a marked by the arrowhead is due to endocytosed VSV. × 325.

type cells with fluorescein, and an anti-Golgi membrane protein antibody (15; kindly provided by Dr. Daniel Louvard) was used to stain both types of Golgi complex with rhodamine. RCA will stain Golgi from wild-type because these have Gal in their bulk glycoproteins; RCA will not stain 15B-derived Golgi complexes because little Gal is incorporated due to the GlcNAc transferase deficiency. Fig. 8 illustrates that the two expected populations of Golgi complex exist both before (Fig. 8a and b) and after (Fig. 8c and d) fusion. Panels a and c are RCA stain; panels b and d are anti-Golgi stain. The Golgi complex remains a perinuclear structure, presumably still associated with the nucleus of its cell of origin. These data seem to rule out a mechanism in which G protein is transferred to wild-type Golgi complex by a fusion of the two types of stacks.

Another double-label immunofluorescence experiment (Fig. 9), now using anti-G antibody (panels b and d) together with RCA (panels a and c) reveals that, after fusion, G protein is associated with wild-type Golgi complex at the light microscope level of resolution. Before fusing mixed monolayers, G-protein antigen is restricted to 15B cells and ricin stain is restricted to wild-type cells (Fig. 9a and b). After fusion (Fig. c and d) G-protein stain is now also found to co-localize with the RCA-staining Golgi from wild-type cells. For this experiment a mutant of VSV (tsG31) with a temperature-sensitive M protein was used to minimize the amount of G protein antigen present (as virions trapped in endocytic compartments by $NH_4Cl$) in wild-type cells before fusion. This mutant (18) is defective in the budding of virions at the restrictive temperature (40°C) but G protein synthesis and transport are unaf-
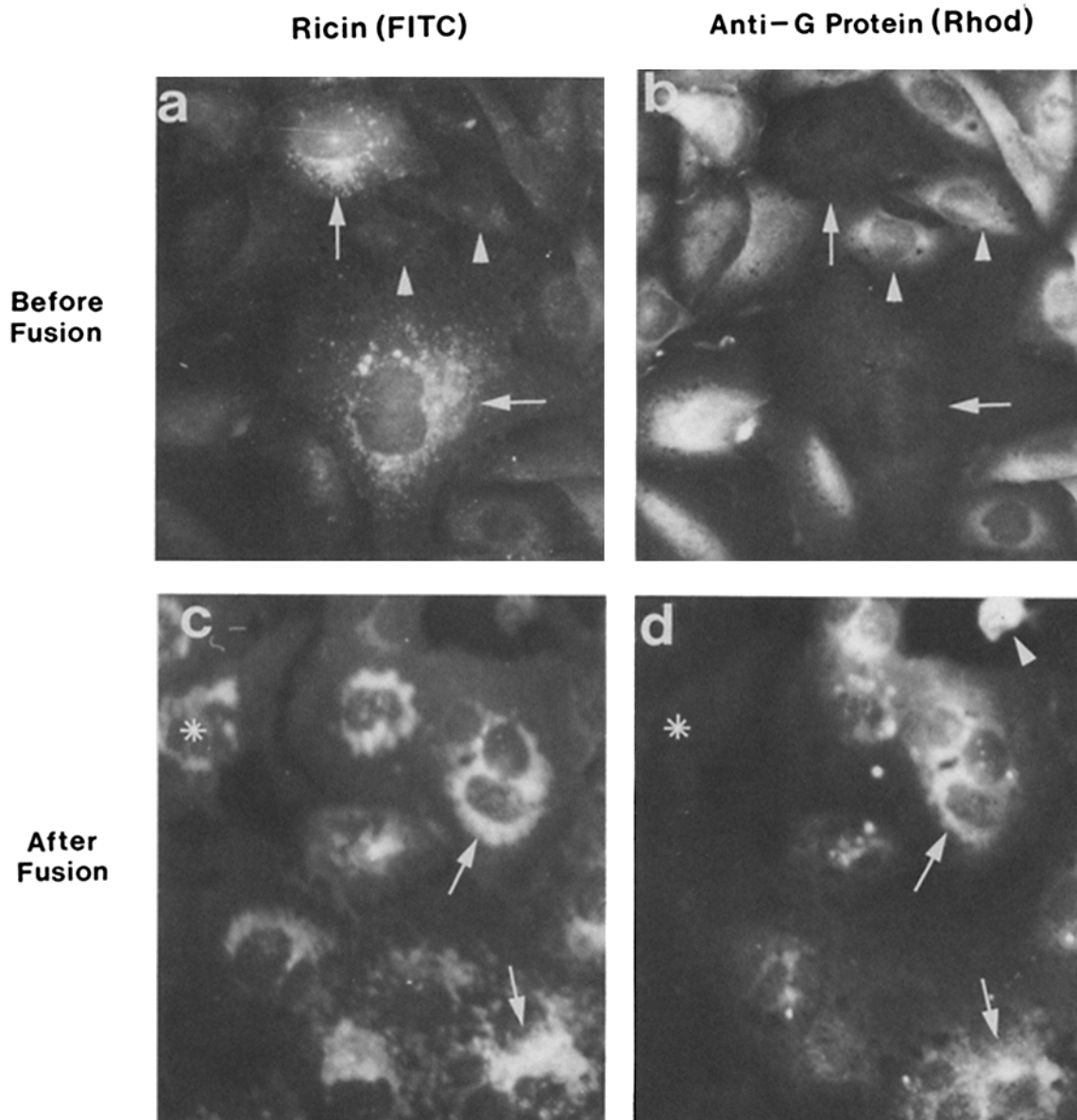
## Ricin (FITC)          Anti−G Protein (Rhod)



FIGURE 9   Appearance of G protein in wild-type Golgi regions after fusion of mixed monolayers (a and c, RCA; b and d, G protein). (a and b) Before fusion. Note that RCA-positive wild-type cells (a, arrows) lack G protein (b, arrows). 15 B cells have G but not RCA sites (arrowheads). The infected 15B cells are generally rounded up resulting in a bright cytoplasmic staining for G. The weak RCA-positive fluorescence seen in some 15B cells is due to virions endocytosed during infection, present in lysosomes. × 130. (c and d) Fixed 20 min after fusion. Note that frequent RCA-positive Golgi complexes (arrows in c) now coincide with staining for G protein (arrows in d). A wild-type cell that remains unfused (asterisk) lacks G protein, as expected. An unfused 15B cell (arrowhead) contains G protein but lacks RCA-binding sites. × 100.

fected (21). Therefore, progeny virions from the infected 15B cells are no longer available for endocytosis by wild-type cells, essentially eliminating their source of G antigen.

## DISCUSSION

This report makes two major points. First, we describe novel processes of transport between cytoplasmic organelles, especially the Golgi complex. Such transport would ordinarily escape detection, but can be revealed when two distinguishable populations of Golgi stacks are introduced into a common cytoplasm by cell fusion. Second, the same population of G-protein molecules that is transferred between Golgi complexes to receive GlcNAc in vivo is also transferred in vitro. G protein that has already been transferred in vivo is not subject to transfer in vitro. This offers strong evidence that the cell-free system (1, 4, 7) is a reconstitution of an authentic segment of protein transport in the Golgi complex. This fact also substantiates the conclusions concerning compartmentation in the Golgi complex reached earlier (7, 20) based on the properties of the cell-free system. Exactly how large a segment of the in vivo transfer process has been reconstituted remains undermined at present.

Fig. 10 summarizes in diagrammatic form our observations and their current interpretation. Within the infected clone 15B cell at least three previously recognized transfers of G protein occur, resulting in successive transfers (Fig. 10a) from RER to Golgi stacks, (Fig. 10b) within and/or across the stack of Golgi cisternae, and (Fig. 10c) out of the Golgi complex and into the plasma membrane.

By introducing a Golgi complex from wild-type cells via fusion, evidence for two new kinds of transfers of G protein has been obtained, namely (Fig. 10d) transfers from the RER of 15B cells to the Golgi complex of wild-type and (Fig. 10e) transfers from the Golgi complex of 15B to the Golgi complex of wild-type. Transfers such as Fig. 10e may occur in our cell-free system; those such as d do not with present conditions. Transfers from plasma membrane to Golgi complexes (the reverse of Fig. 10c) do not occur either in vivo or in vitro, as would be expected if process c were vectorial.

The main evidence for an inter-Golgi transfer (Fig. 10e) reported here is that freshly acylated G protein is transferred to exogenous Golgi complexes. Electron microscopy, cell fractionation, and the kinetics of acylation (2, 4, 19, 24) make it very likely that this pool of G protein is in the Golgi complex. For example, the population of G protein in CHO 15B cells that is pulse-labeled with [³H]palmitate co-fractionates with Golgi markers (such as mannosidases I and II and GlcNAc transferase II) on sucrose gradients (4, 5). Also, these same [³H-palmityl]G protein molecules have mannose₅-oligosaccharides attached (4, 7) indicative of the action of Golgi mannosidase I. Unfortunately, the responsible acyltransferase has not yet been identified or localized directly. The definitive evidence for inter-Golgi transfers such as Fig. 10e is presented in the following article (23) which shows that G protein, tagged in one Golgi population by GlcNAc transferases, can be further glycosylated in a second Golgi population by Gal and sialyl transferases.

Transfer of G protein present in the RER of 15B cells (at the time of fusion) to wild-type Golgi complex after fusion could either occur directly as envisioned in Fig. 10 (arrow d) or instead indirectly via the 15B Golgi complex (arrow a followed by arrow e). Our data favors the existence of a more direct transfer pathway (Fig. 10d) because transfer from RER
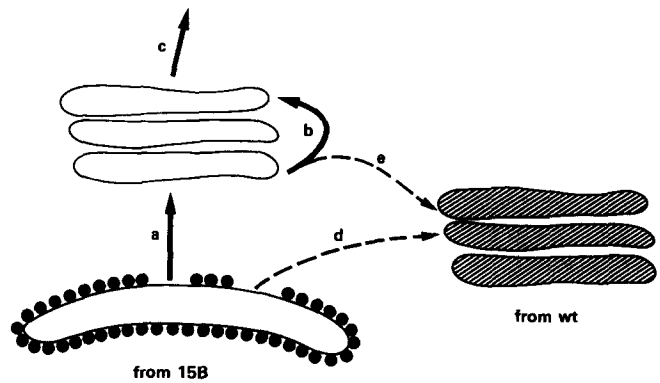


FIGURE 10  Illustration of the kinds of intercompartmental transfers that can occur before and after cell fusion. The RER and Golgi stacks on the left are from infected 15B cells; the shaded Golgi on the right is from uninfected wild-type cells and contains GlcNAc transferase I missing from the mutant Golgi complex on the left. Before fusion, successive transfers such as a, b, and c result in movement of G protein from RER to Golgi complex, through the Golgi stack, and into the plasma membrane, respectively. After a distinguishable population of Golgi complexes from wild-type cells is introduced by fusion, now transfers such as d and e can be detected. Transfer d is proposed to occur by the same process as transfer a; transfer e would occur by the same mechanism as transfer b. Therefore, transfer within a Golgi stack (b) would occur by a dissociative mechanism (e), such as budded transport vesicles.

is about twice as efficient as transfer from Golgi (for example, compare Fig. 3, 0-min chase with 10-min chase).

The processes, such as the transfers in Fig. 10d and e that result in movement between organelles initially present in different cytoplasms, are presumably made possible by the same transport mechanisms that operate before cell fusion. Supporting this view is the fact, shown in the next article (23), that inter-Golgi and intra-Golgi transport occur at the same rates, and are unaffected by the act of fusion. An inter-Golgi transfer (such as in Fig. 10e) would then result from the same molecular mechanism as a corresponding intra-Golgi transfer (such as in Fig. 10b); the difference being that in an inter-Golgi transfer the destination happens to be in a different stack.

The existence of dissociative transfer processes such as Fig. 10d and e would be most compatible with the view that transport between RER and the Golgi complex and between cisternae of the Golgi stack is carried out by diffusible transport vesicles. The rate and efficiency with which G protein can be transferred between organelles residing inside different cells moments before fusion is surprising. It suggests that, to a first approximation, a vesicle budding from ER or from a Golgi cisterna can choose its specific target (such as another Golgi cisterna) more or less at random, without regard to physical proximity, from among those biochemically suitable membranes that are present in the cell. If so, then the specificity of vesicular traffic would have to be dictated by the intrinsic properties of vesicles and their targets and not influenced by the physical arrangement or juxtaposition of organelles in the cytoplasm. In other words, these transport processes can function in cells as if the organelles involved were in a random suspension, as in a reconstitution experiment.

out how the pH-triggered fusion could be used in the present context. We thank John Lenard for his comments on this and the following article. Also, we are indebted to Helen Blau for the generous use of her fluorescence microscope, Daniel Louvard and Doug Lyles for kind gifts of antibodies, and Darrell Dobbertin for the electron microscopy.

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