Effect of Microtubule Assembly Status on the Intracellular Processing and Surface Expression of an Integral Protein of the Plasma Membrane

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ABSTRACT We studied the effects of changes in microtubule assembly status upon the intracellular transport of an integral membrane protein from the rough endoplasmic reticulum to the plasma membrane. The protein was the G glycoprotein of vesicular stomatitis virus in cells infected with the Orsay-45 temperature-sensitive mutant of the virus; the synchronous intracellular transport of the G protein could be initiated by a temperature shift-down protocol. The intracellular and surface-expressed G protein were separately detected and localized in the same cells at different times after the temperature shift, by double-immunofluorescence microscopic measurements, and the extent of sialylation of the G protein at different times was quantitated by immunoprecipitation and SDS PAGE of [^{35}S]methionine-labeled cell extracts. Neither complete disassembly of the cytoplasmic microtubules by nocodazole treatment, nor the radical reorganization of microtubules upon taxol treatment, led to any perceptible changes in the rate or extent of G protein sialylation, nor to any marked changes in the rate or extent of surface appearance of the G protein. However, whereas in control cells the surface expression of G was polarized, at membrane regions in juxtaposition to the perinuclear compact Golgi apparatus, in cells with disassembled microtubules the surface expression of the G protein was uniform, corresponding to the intracellular dispersal of the elements of the Golgi apparatus. The mechanisms of transfer of integral proteins from the rough endoplasmic reticulum to the Golgi apparatus, and from the Golgi apparatus to the plasma membrane, are discussed in the light of these observations, and compared with earlier studies of the intracellular transport of secretory proteins.

There is good evidence that, during the intracellular transport of secretory and membrane components, the transfer of these components from the Golgi apparatus (GA)[1] to the cell surface occurs by way of specific vesicles that bud off the *trans* face of the GA and migrate to the plasma membrane (for a recent review, see reference 13). It is thought that a similar process occurs to transfer these secretory and membrane components from their sites of synthesis and insertion in the rough endoplasmic reticulum (RER) to the GA, with so-called "transition vesicles" budding off the RER and migrating to the *cis*-face of the GA. The fact that in most interphase cells the GA is a compact organelle confined to a region near the nucleus, whereas the RER is generally widely distributed throughout the cytoplasm, raises a number of geometrical questions about such putative "transition vesicles" and how they arrive at the *cis* face of the GA. Do such vesicles arise from sites in the RER that are randomly distributed in the cytoplasm? If so, do the vesicles then simply diffuse to their destinations? Or are they directed along tracks, as, for example, might be provided by elements of the cytoskeleton? The experiments described in this paper were carried out to determine whether microtubules play a role in the intracellular transport, and

[1] *Abbreviations used in this paper:* GA, Golgi apparatus; NRK, normal rat kidney; O-45, temperature-sensitive mutant of vesicular stomatitis virus Orsay-45; RER, rough and endoplasmic reticulum; VSV, vesicular stomatitis virus.

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presumed vesicular transfers, of an integral membrane protein from the RER to the plasma membrane. A great many studies have been carried out on the effect of drug-induced microtubule disassembly on the secretion of proteins and other secretory components (see Discussion), but much less has been done with membrane proteins. Furthermore, the results of these studies have been conflicting.

For the study of a specific integral protein of the plasma membrane, a convenient model system is the G protein of vesicular stomatitis virus (VSV) in VSV-infected cells. The G protein in these cells is processed intracellularly and transported to the cell surface just as are the integral membrane proteins of the cell (27). The intracellular transport of the G protein from the RER to the plasma membrane can be synchronized by the use of a temperature-sensitive mutant of VSV, Orsay-45 (O-45) (26), and a temperature shift-down protocol (4, 41).

We have performed two types of experiments with this system, thereby examining the effects of changes in microtubule assembly status upon the expression and properties of the G protein. The first type of experiment involved immunofluorescent labeling for the G protein as it moved through the cell interior to the cell surface after the temperature shift-down. The second type examined the state of oligosaccharide processing of the G protein as it moved to the cell surface. Knipe et al. (19) have shown that the G protein exists in two cellular forms, G_1, and G_2, in addition to the nonglycosylated primary translation product, G_0. G_1 and G_2 have different electrophoretic mobilities in SDS PAGE which is attributable to the presence of sialyl residues on G_2 that are not present on G_1. The additions of terminal sugars, such as sialic acid, are Golgi-associated glycosylation events (18, 19). We therefore investigated whether microtubule assembly status had any quantitative influence on the time course of the $G_1 \rightarrow G_2$ conversion, after the temperature shift-down.

Both types of experiments showed that profound changes in the state of microtubule assembly and distribution have no detectable effects on the rates of G protein intracellular transport from the RER through the Golgi complex, or on sialic acid incorporation; furthermore, by immunofluorescence, there was also no measurable effect on the rate of surface expression of the G protein in O-45-infected cells. These results are consistent with an earlier finding (17) that the overall rate of production of infective VSV by cultured cells was not significantly altered by colchicine or colcemid treatment. These are particularly striking conclusions, in view of the fact that the changes in microtubule assembly status that we examined resulted in profound changes in the overall configuration and intracellular locations of elements of the Golgi complex, as in documented in the preceding article (38), and has earlier been observed by others (10, 29, 36). However, although the rate of the surface expression of the G protein was not affected qualitatively, the *polarity* of the surface expression was altered by changes in microtubule assembly status and the intracellular disposition of the GA.

MATERIALS AND METHODS

Cell Culture and Virus Infection: Normal rat kidney (NRK) cells were cultured and infected with the O-45 mutant of VSV as previously described (38). For infection experiments cells were cultured either on glass coverslips or 7.5% gelatin films. It was found that cells grown on the gelatin films were infected at significantly higher levels (60–70%) compared with those grown on untreated glass coverslips (40%). This approach to cell infection was therefore adopted for the biochemical studies.

Manipulation of Cytoplasmic Microtubule Assembly Status and Positioning of Golgi Elements: Nocodazole treatments for the depolymerization/repolymerization of the cellular microtubules accompanied by the dispersion/recompaction of the Golgi elements, as well as taxol treatments leading to the reorganization and bundling of microtubules and consequent redistribution of Golgi elements, followed exactly the procedures in the accompanying paper (38). In one additional series of experiments, the O-45-infected cells were treated with nocodazole at 30 μM for 90 min at 39.9°C to disassemble the microtubules, and then cytochalasin D was added at a final concentration of 4 μM to impair microfilament function, before shifting the cells to 32°C. In another series, cytochalasin D was used without nocodazole before the temperature shift-down.

Radiolabeling of O-45–VSV-infected Cells with [^{35}S]Methionine: Infected NRK cultures on glass coverslips were transferred to tubes containing methionine-free Dulbecco's modified Eagle's medium buffered with 15 mM HEPES, pH 7.4, and were placed in a 39.9°C circulating water bath. A stir bar was added to each tube to insure adequate temperature equilibration. After a 90-min incubation period at 39.9°C, nocodazole (final concentration 30 μM) or taxol (10 μM) and [^{35}S]methionine (50 μCi/ml) (New England Nuclear, Boston, MA) were added. Incubations were continued for another 90 min at 39.9°C. Afterwards, 1 mM unlabeled methionine was added before the transfer of tubes to a 32°C circulating water bath, to chase the methionine-labeled G protein out of the RER. Coverslip cultures were processed for immunoprecipitation of [^{35}S]methionine-labeled viral proteins at zero time, 13, min and 30 min after the shift to 32°C.

Immunoprecipitation of [^{35}S]Methionine-labeled VSV Proteins and Digestion with Neuraminidase: Immunoprecipitation was carried out by using a multispecific rabbit antiserum raised to purified and ultraviolet-inactivated VSV, and containing antibodies recognizing the G, N, NS, and M proteins of the virion (3). The IgG fraction of this antiserum was purified by DE-52 chromatography (Whatman Chemical Separation Inc., Clifton, NJ).

Coverslip cultures were prepared for immunoprecipitation by three rapid rinses with ice-cold PBS followed by lysis with 100 μl of 1% SDS in PBS, pH 7.4, containing 1 mM phenylmethylsulfonyl fluoride, 0.1% aprotinin, and DNase at 20 μg/ml for 1–2 min at room temperature. The cell lysate was then transferred to an Eppendorf tube and heated at 100°C for 5 min. It was then diluted with 1% Nonidet P-40/1% deoxycholate in PBS to 0.1% in SDS. Insoluble material was pelleted at 12,000 *g* in a SS-34 Sorvall rotor (DuPont Instruments, Sorvall Biomedical Div., Newton, CT). To the recovered supernatant was added 100 μl of the purified IgG fraction of rabbit antibodies directed to VSV proteins. Incubation was continued for 90 min at 0–4°C. Afterwards, 100 μl of a slurry of Protein A–Sepharose 6MB beads (Pharmacia Fine Chemicals, Piscataway, NJ) prewashed with 0.1% SDS/1% Nonidet P-40/1% deoxycholate was added and further incubated for another hour at 0–4°C with gentle agitation. The beads were spun down at 1,000 *g* for 5 min, then washed three times with PBS, once with PBS containing 0.5 M NaCl, and finally with 0.06 M Tris-HCl, pH 8.8. Each bead pellet was taken up in sample buffer containing dithiothreitol and subsequently alkylated with iodoacetamide (23). Neuraminidase digestion of immunoprecipitates was performed by washing reacted Protein A–Sepharose beads as above but replacing the final wash of Tris buffer with 0.05 M Na acetate, pH 5.5, 154 mM NaCl, 4 mM $CaCl_2$. The beads were then incubated with 100 μl of neuraminidase, *Vibrio cholerae* (Calbiochem-Behring Corp., San Diego, CA) and incubated at 37°C for 30 min. The reaction was terminated by rinsing the beads one time with 0.06 M Tris-HCl, pH 8.8, before preparation for electrophoresis.

SDS PAGE: Solubilized [^{35}S]methionine-labeled immunoprecipitates were electrophoresed in 10% polyacrylamide slab gels by the method of Laemmli (22). Gels were allowed to run an additional hour after the tracking dye reached the bottom of the gel to maximize resolution of the viral proteins, particularly the two cellular forms of the G protein (18, 19). The gels were stained with Coomassie Brilliant Blue, destained overnight and then processed for fluorography with EN^{3}HANCE (New England Nuclear). Gels were then dried down and exposed to Kodak BB-5 x-ray film (Eastman Kodak Co., Rochester, NY) and developed at −70°C. Selected autoradiograms were scanned on a Joyce–Loebl microdensitometer (Vickers Instrument Co., London, England).

Surface and Intracellular Expression of the G Protein as Detected by Double-Label Immunofluorescent Microscopy: For immunofluorescent studies, affinity-purified guinea pig and rabbit antibodies to the G protein of VSV were prepared as described (3, 37, 38). Cells were routinely cultured on glass coverslips. All other manipulations exactly paralleled those performed for the radiolabeling studies. Three time points were selected for simultaneous labeling of the G protein on the cell surface and

intracellularly. The zero time point was the time just before the transfer of cultures to the 32°C permissive temperature. Two additional time points were selected, 13 and 30 min (2, 4). At these times, coverslips cultures were fixed with 3% paraformaldehyde in PBS for 30 min at room temperature. After three rinses in PBS/0.1 M glycine cells were labeled for 30 min for surface-associated G protein with the affinity-purified guinea pig antibody directed to the G protein. After three 10-min rinses in PBS/0.1 M glycine, the cells were then permeabilized for 5 min with 0.1% Triton X-100 in PBS, rinsed quickly three times with PBS/0.1 M glycine and finally incubated for 30 min with the rabbit affinity-purified antibody directed to the G protein. This was followed by three additional rinses in PBS/0.1 M glycine and finally incubation with a mixture of cross-absorbed fluoresceinated-goat anti-rabbit IgG and rhodamine-conjugated goat anti-guinea pig IgG, each at 10–15 μg/ml. After three more 10-min rinses in PBS/0.1 M glycine, immunolabeled cultures were examined with a Zeiss Photomicroscope III (Carl Zeiss, Inc., New York) equipped with a vertical illuminator RSII for epifluorescence and with transmitted light for Nomarski differential interference contrast. All observations were made with a × 63 numerical aperture 1.4 Planapochromat oil objective (Carl Zeiss, Inc.). Preparation of the affinity-purified primary and secondary antibodies were as described (2, 38).

RESULTS

Surface and Intracellular Expression of the G Protein Synthesized in Cells with an Altered Microtubule Assembly Status

The time course of the intracellular and surface-associated expression of the G protein after the shift from 39.9°C to 32°C was studied qualitatively by indirect double-label immunofluorescent microscopy, using affinity-purified guinea pig anti-G protein for surface staining of nonpermeabilized cells, followed by intracellular labeling in 0.1% Triton X-100–permeabilized cells with affinity-purified rabbit anti-G protein. Cultures of O-45-infected cells were treated for the last 90 min at 39.9°C with either 30 μM nocodazole or 10 μM taxol, then transferred to the permissive temperature of 32°C, whereupon the G protein moved synchronously out of the RER on its way to the cell surface. In the composite of Fig. 1, the cellular status of the G protein (surface and intracellular) in a representative population of O-45-infected cells is shown, the top row of cells with an intact microtubule system, and the bottom row of cells with microtubules completely disassembled by nocodazole treatment. At the zero time point just before the temperature shift to 39.9°C, the intracellular distribution of the G protein was indistinguishable in cells with (Fig. 1 *A/A'*) or without intact microtubules (Fig. 1 *B/B'*). A diffuse cytoplasmic staining, apparently representing the G protein in the endoplasmic reticulum, along with a distinct nuclear envelope staining, was evident as previously noted (4). No zero-time staining for the G protein could be detected on the cell surface for either untreated or nocodazole-treated cells (Fig. 1, *A'* and *B'*), except for the occasional staining of virus remaining on the cell surface after washing before the first incubation at 39.9°C.

At the 13-min time point, the G protein had accumulated in the Golgi complex inside untreated cells (Fig. 1 *C*), as has been observed earlier (3, 4). In 13-min cells whose microtubules were completely disassembled, the G protein appeared in discrete units which were no longer confined to the perinuclear region, but instead were dispersed throughout the cytoplasm (Fig. 1 *D*). These G protein-labeled units were shown to be elements of the Golgi complex (see Discussion). Surface labeling of the G protein was not yet evident at 13 min in either control (Fig. 1 *C'*) or nocodazole-treated (Fig. 1 *D'*) cells.

At the 30-min time point the intracellular G protein was found not only in the Golgi complex but also in other regions of cytoplasm (Fig. 1, *E* and *F*). At this time, the G protein had appeared at the cell surface in both control (Fig. 1 *E'*) and nocodazole-treated (Fig. 1 *F'*) cells. In the untreated cells whose Golgi complex showed a compact configuration in the perinuclear region (arrowhead, Fig. 1 *E*), the G protein on the cell surface generally exhibited a marked nonuniform distribution, concentrated over that region of the surface that faced the Golgi complex (Fig. 1 *E'*, arrows). Such a markedly polarized surface appearance of the G protein was not seen in isolated cells whose microtubules were disassembled and Golgi elements dispersed. Occasionally, however, where two cells were in contact, the contact regions showed a concentration of surface labeling of the G protein (Fig. 1 *E'*, open arrows), possibly due to some cell–cell capping effect after surface expression.

In taxol-treated cells, microtubules were free of the microtubule-organizing center and bundled into a variety of configurations (9, 38) (Fig. 2, *B* and *D*). Under such circumstances, the Golgi elements were not randomly dispersed in the cell periphery as in the nocodazole-treated cells, but were present in discrete intracellular locations near one of the two end regions of the microtubule bundles (compare Fig. 2 *C*, arrows, with *D*). In taxol-treated O-45-infected cells 30 min after the temperature shift-down, G protein appeared at the cell surface at this time (Fig. 2 *A*), and often showed a polarized distribution.

In another set of experiments, the combined effects of microtubule disassembly by nocodazole and the impairment of microfilament functions by cytochalasin D, on the surface expression of the G protein 30 min after the temperature shift-down, was examined. As is clear in Fig. 3 *B*, such treatment had no marked effect on the intensity of surface expression of the G protein. Treatment with cytochalasin D alone had a profound effect on cell morphology (Fig. 3 *C*), but no appreciable effect on surface G-protein appearance (Fig. 3 *D*).

Sialylation of the G Protein in Cells with an Altered Microtubule Assembly Status

Sialylation of the G protein was monitored by immunoprecipitation of [^{35}S]methionine-labeled viral proteins from O-45-infected cells at 0, 13, and 30 min after the temperature shift-down. In untreated cells, the G protein showed a progressive shift from the G_1 to the slower-migrating G_2 form (Fig. 4, lanes *A–C*), the G_2 form being convertible to G_1 by neuraminidase treatment (lane *D*). In nocodazole-treated cells (lanes *E–H*), in cells recovered from nocodazole treatment (lane *I*), and in taxol-treated cells (lane *J*), the conversion to the G_2 form was not detectably different in rate or extent from normal. This is demonstrated quantitatively in Table I, which gives the results of the analysis of densitometric scans of the autoradiograms in Fig. 4, and shows that the ratio of G_2 to the N/NS viral proteins was the same in all cases within experimental error.

DISCUSSION

The experiments that we have carried out on the effect of microtubule assembly status on the intracellular transport and surface expression of the G protein of VSV can most conveniently by analyzed by considering two stages of the process: first, the transport from the RER (where the G protein is present before the temperature shift-down) through the

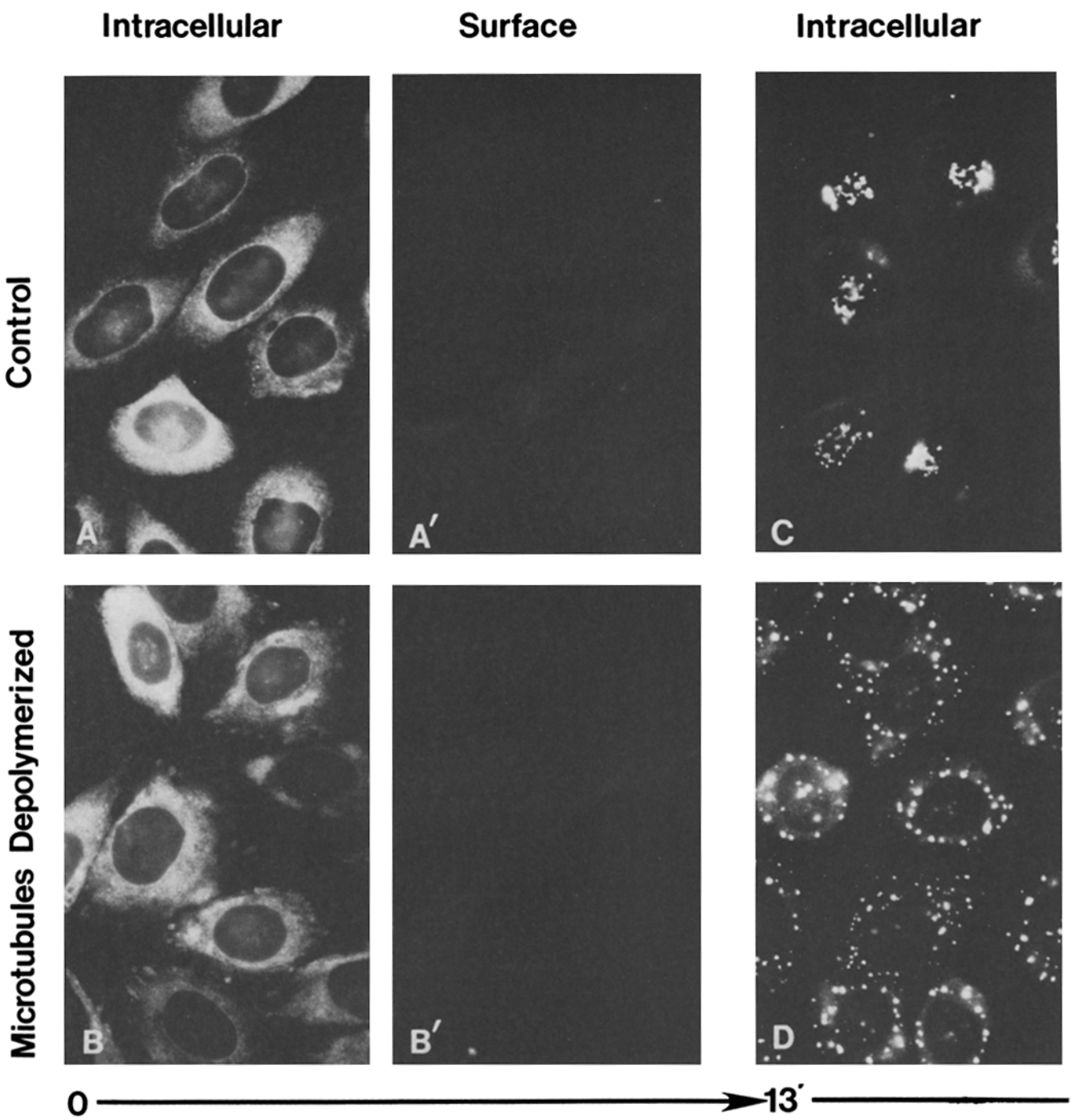

FIGURE 1 NRK cells infected with the O-45 temperature-sensitive mutant of VSV and examined either just before the temperature shift-down to 32° (zero time, *A*/*A*′ and *B*/*B*′), 13 min after the shift (*C*/*C*′ and *D*/*D*′), or 30 min after the shift (*E*/*E*′ and *F*/*F*′). The top row of cells was controls; the bottom row of cells was treated with nocodazole to completely disassemble the cytoplasmic microtubules. The cells were double-immunofluorescent labeled for the intracellular G protein (*A*, *B*, *C*, *D*, *E*, and *F*) and for their surface-expressed G protein (*A*′, *B*′, *C*′, *D*′, *E*′ and *F*′, respectively) (see text for methods and analysis of results). The arrowheads in *E* point to perinuclear GAs labeled with G protein. On the surfaces of the corresponding cells, the first expression of the G protein is seen to be polarized (arrows), and juxtaposed to the GA inside the cell. No such surface G protein polarization is seen on the nocodazole-treated cells (*F*′). × 790.

Golgi complex to the stage where it is sialylated; and second, the transport from the GA to the cell surface.

Transfer of the G Protein from the RER through the GA

The attachment of sialyl and other terminal sugars of the oligosaccharide chains of the G protein occurs in one or more of the saccules on the *trans* side of the Golgi complex (13). Our results indicate that neither complete disassembly of the cytoplasmic microtubules by nocodazole, nor the drastic reorganization of the microtubules after taxol treatment, has any significant quantitative effect on the rate or extent of sialylation of G protein after the temperature shift-down (Fig. 4, Table I). By immunofluorescence observations of nocodazole-treated cells at 13 min after the temperature shift, G

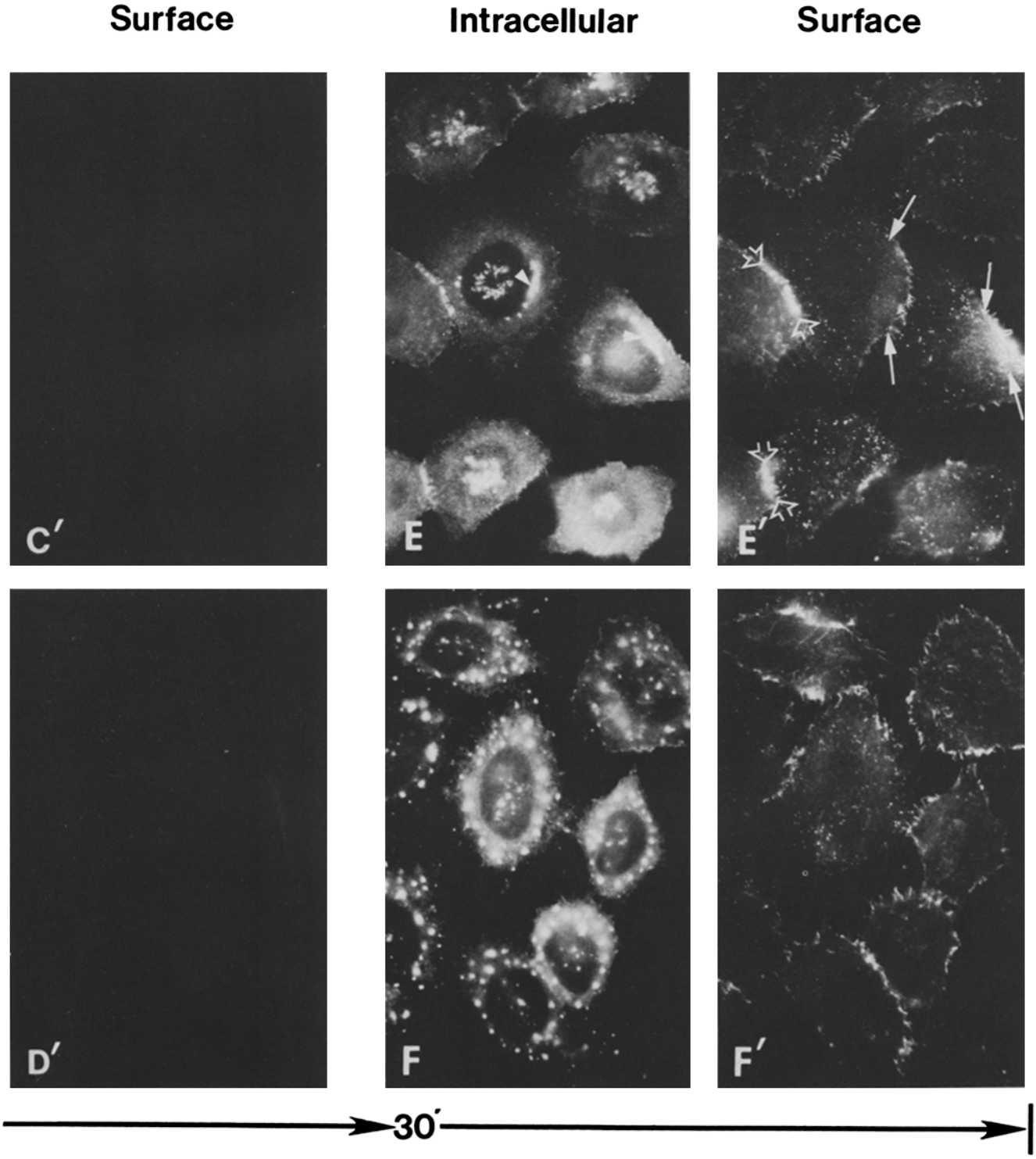

FIGURE 1

protein was found in discrete units that were widely separated in the cell periphery (Fig. 1 *D*). These dispersed units must represent elements of the GA that were functionally intact, in that oligosaccharide processing of the G protein was apparently normal in these cells. That these units were elements of the GA was further demonstrated by double-labeling of nocodazole-treated O-45-infected cells with the antibodies to the G protein and with the anti-Golgi antibodies of Louvard et al. (29) (Rogalski, A. A., G.-A. Keller, and S. J. Singer, unpublished observation). These results correspond to earlier electron microscopic observations that elements of the Golgi complex are dispersed into the cell periphery upon microtubule disassembly (10, 29, 36). In taxol-treated cells, the G protein-labeled (and functionally intact) GA elements were associated with the end regions of microtubule bundles that were arranged in widely different distributions from one cell to another (38).

Two conclusions follow from these findings: (*a*) the transfer of the G protein from its original uniform dispersion throughout the RER into the GA, as well as the transfer through the GA, is independent of microtubules; and perhaps more significant, (*b*) these transfers are not affected by radically differ-

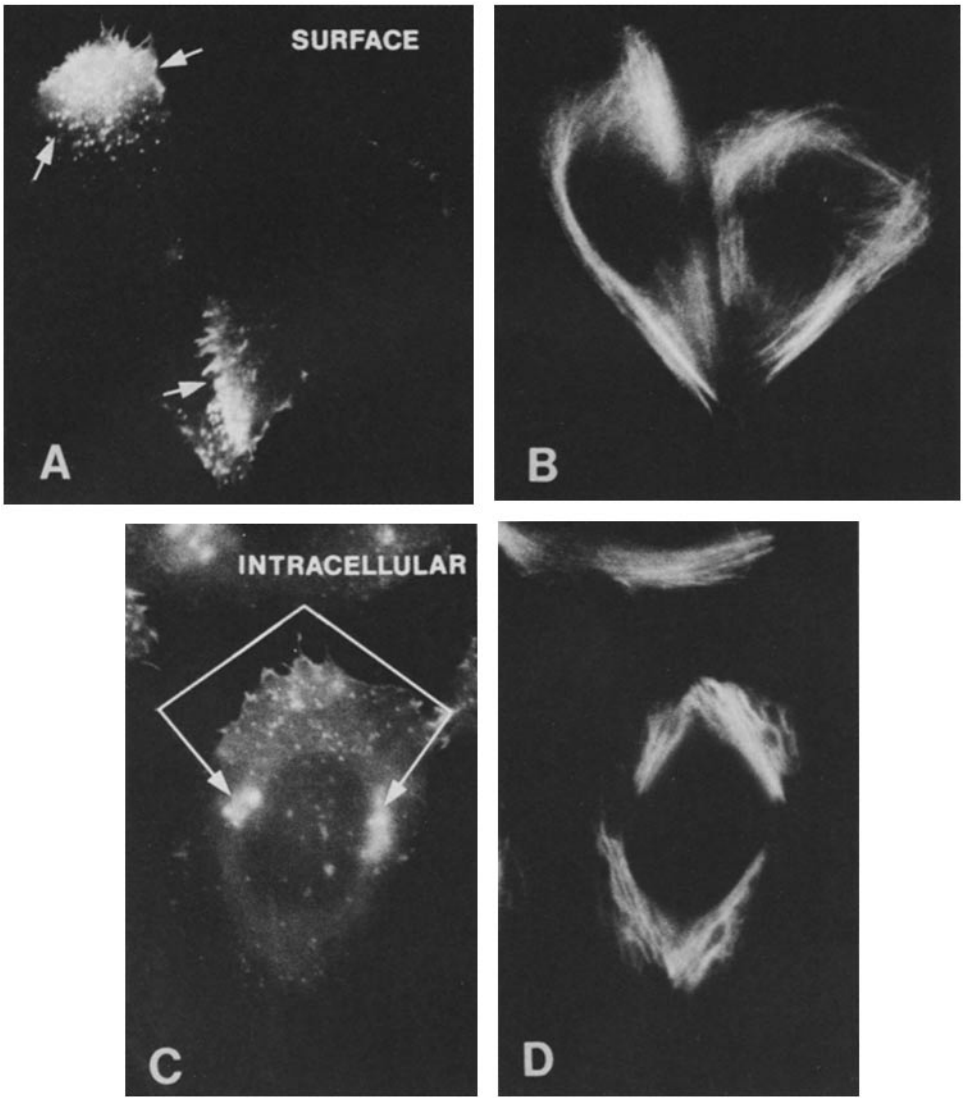

FIGURE 2 O-45-infected NRK cells treated with taxol and examined 30 min after the temperature shift-down. For the pair A and B, double-immunofluorescent labeling of the cells for surface G protein (*A*) and for intracellular microtubules (*B*) was carried out. For the pair *C* and *D*, intracellular G protein (*C*) and the microtubules (*D*) were immunolabeled. The arrows in C point to the G protein-labeled elements of the GA that are located near the end regions of the bundles of the free microtubules in the cell. The arrows in *A* point to the polarized surface location of the G protein juxtaposed to the end regions of the microtubule bundles inside the cell. × 665.

ent geometrical arrangements of elements of the GA in the cell. In other experiments with O-45-infected cells, we have recently shown that, starting from an initially uniform distribution of the G protein throughout the RER at the nonpermissive temperature (4), the G protein enters uniformly and fairly synchronously at one face (probably the *cis* face) of the stacks of GA saccules by 3 min after the shift to 32°C (3). From the experiments reported in this paper, the implication is that in nocodazole- or taxol-treated cells, this rapid and synchronous entry of the G protein into the Golgi stacks of saccules also applies, despite the radically altered geometrical arrangement of the GA elements in the cell.

These results bear on the detailed mechanisms by which the transfer of integral membrane proteins and secretory proteins (see below) occurs from the endoplasmic reticulum to the Golgi complex. This transfer is now generally thought to be mediated via "transition vesicles" that bud off the RER and are transported to, and fuse with, elements of the Golgi complex (13). One possibility to consider is that the transition vesicles form at membrane sites that are randomly distributed throughout the RER. If so, how do the vesicles get to the *cis* face of the GA stacks? Our experiments involving microtubule disassembly, coupled with cytochalasin D-induced impairment of microfilament function, argue against the idea that such vesicles are directed to the GA along cytoskeletal tracks. Do the vesicles then diffuse from their random sites of origin to the *cis* faces of the GA elements? Although we cannot rule out this possibility, it seems unlikely, because to account for the synchrony of G-protein appearance at the *cis* face of the GA (3), and the fact that the rate of that appearance is independent of the geometry of the GA elements in the cell, diffusion of the vesicles would always have to be non-rate limiting for the transfer process. Yet this transfer takes <3 min at 32°C (3).

Another possibility to consider is that the transfer of G protein occurs by way of specific (rather than random) mem-

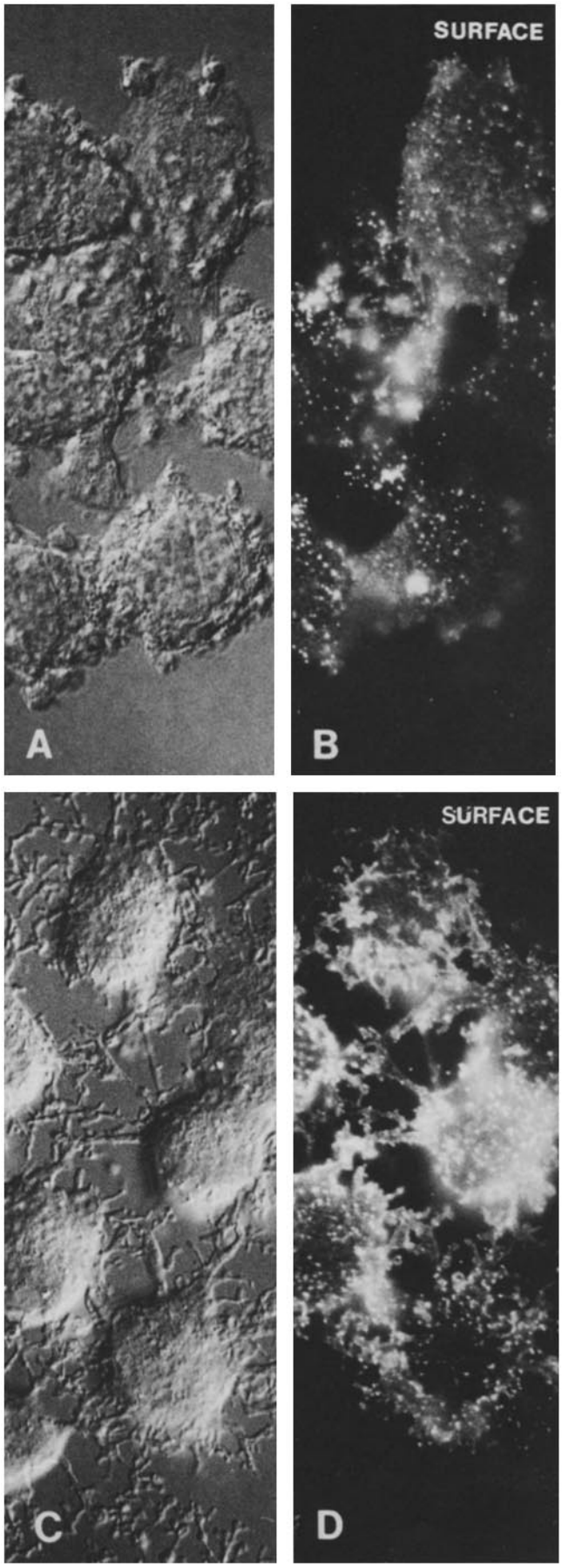

FIGURE 3 O-45-infected NRK cells treated with nocodazole and cytochalasin D (the pair *A* and *B*), or cytochalasin D alone (*C* and *D*), then examined 30 min after the temperature shift-down for their surface expression of the G protein (*B* and *D*). The Nomarski images of the cells are shown in *A* and *C*. × 935.

brane sites in the RER that are indirectly connected to, or are even directly continuous with, *cis* saccules of the GA elements. Such RER–GA connections or continuities might then be maintained upon nocodazole- or taxol-induced changes in the dispersion of the Golgi complex. Clearly, if specialized membrane or indirect connections did indeed exist between the RER and the *cis* face of the GA stacks, transition vesicles might form at these connections. On the other hand, if continuities exist between the RER and GA, transition vesicles need not be involved at all in this transfer process. In this context, it is perhaps relevant that in our immunoelectron microscopic studies of the RER–GA transfer of the G protein (3), we obtained no clear evidence for the appearance of G protein-labeled transition vesicles before the entry of G into the *cis* saccules of the GA. Furthermore, a number of investigators (6, 11, 14, 15, 25, 30, 31) have provided electron microscopic evidence for the existence of continuities between the RER and GA, although the matter is still considered controversial (13). It is obvious, however, that the results discussed in this paper could readily be explained by a model that involved RER–GA transfers at specialized RER sites that were maintained proximal to, or were continuous with, the *cis* saccules of the GA. It is also obvious that our results do not prove such a model. We suggest that the possible existence of specific types of functional connections between the RER and the *cis* saccules of the GA deserves further consideration and additional experimental investigation.

Transfer of the G Protein from the GA to the Plasma Membrane

The transfer of the G protein to the plasma membrane was monitored by immunofluorescence measurements of the surface appearance of the protein 30 min after the temperature shift-down. By these measurements, we did not observe any marked delay in the surface expression of the G protein nor any striking changes in the amounts expressed in cells with altered microtubule assembly status. Therefore, despite the qualitative nature of these immunofluorescence results, the indications are that intact cytoplasmic microtubules in their normal association with the microtubule-organizing centers are not required in order for the transfer of G protein from the GA to the plasma membrane to occur. These results at the individual cell level correspond to the earlier finding (17) that the yields of infective VSV were not detectably altered by treatment of infected cells with colchicine or colcemid.

Changes in microtubule assembly status did have significant effects, however, on the polarity of the surface expression of G. This polarity was correlated with the distribution of GA elements inside the same cells. Where the GA elements were collected in the normal perinuclear compact configuration (Fig. 1*E*) the G protein was first observed on those regions of the cell surface that were directly apposed to the GA elements (Fig. 1*E'*). In taxol-treated cells, GA elements were nonrandomly distributed in the cytoplasm (Fig. 2*C*), and the surface expression of the G protein was generally polarized (Fig. 2*A*). When the GA elements were randomly dispersed, however, as in nocodazole-treated cells (Fig. 1*F*), the surface appearance of G on isolated cells was essentially uniform (Fig. 1*F'*).

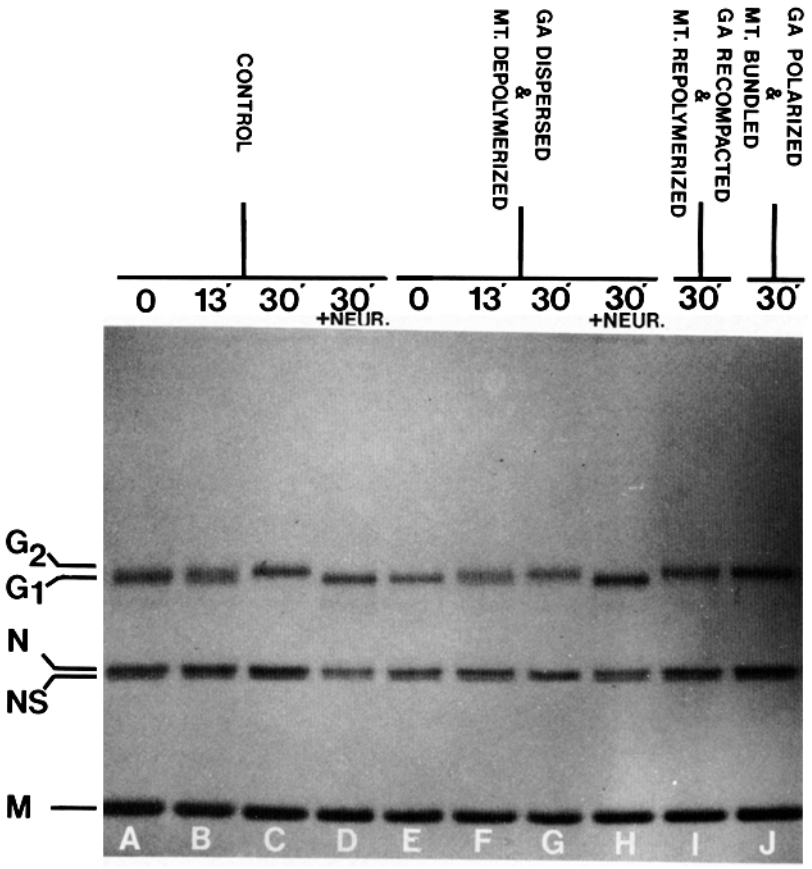

FIGURE 4 SDS PAGE of immunoprecipitates of the viral proteins from extracts of [^{35}S]methionine-labeled, O-45-infected NRK cells. Lanes *A–C* are from control cells 0, 13, and 30 min after the temperature shift-down; lane *D* is of the 30-min control cells after neuraminidase treatment of the extract, showing that sialylation of the G_2 form of the G protein distinguishes it from the G_1 form. *N*, *NS*, and *M* are the other viral proteins. Lanes *E–G* are of similar experiments with cells that had been first treated with nocodazole before the temperature shift-down, with lane *H* showing the corresponding neuraminidase-treated 30 min extract. Lane *I* is of nocodazole-treated cells that had been washed free of the drug and the microtubules allowed to repolymerize before the temperature shift, examined 30 min after the shift. Lane *J* is of taxol-treated cells 30 min after the shift. At the 30 min time point (lanes *C*, *G*, *I*, and *J*) all of the differently treated cells showed essentially the same degree of G_2 formation.

TABLE I

Relative Amounts of VSV Proteins Synthesized by 30 min after Temperature Shift

Condition	Ratio of G_2:N/NS:M
Control	0.76:1.0:1.31
Microtubules disassembled; GA dispersed (nocodazole)	0.86:1.0:2.10
Microtubules reassembled; GA recompacted (nocodazole washout)	0.82:1.0:1.77
Microtubules bundled; GA dispersed and polarized (taxol)	0.70:1.0:1.44

The difference in the polarity of surface expression of G in cells with intact microtubules compared to cells with disassembled microtubules has, we believe, important consequences. We have recently provided evidence (21) that, in cells undergoing directed migration, the compact GA and microtubule organizing center are coordinately positioned forward of the nucleus in the direction of cell migration; insertion of new membrane mass is accordingly directed to the leading edge of the motile cell, and serves as a critical factor in cell extension (2). The well-known fact that cell migration is inhibited by microtubule disassembly can be interpreted in the light of our present experiments as due, not necessarily to a requirement for force generation by microtubules, but rather to a dispersion of the GA elements inside the cells that accompanies microtubule disassembly, and a consequent loss of the polarity of insertion of new membrane mass. In other words, if new membrane mass is inserted uniformly over the entire cell surface, no leading edge can develop. Another phenomenon for which these considerations are relevant involves the cytotoxic activity of natural killer cells and cytotoxic lymphocytes towards their bound target cells. There is evidence that this cytotoxic activity involves the secretion by natural killer or cytotoxic lymphocyte effector cells of cytotoxic elements into the membrane of the target cells where the two cells are in contact (12, 32). In cell couples containing one effector cell and one target cell, it has been demonstrated that the compact GA and microtubule organizing center are oriented towards the bound target cell, both with natural killer cells (22) and cytotoxic lymphocytes (16). In cases where nocodazole-induced disassembly of microtubules in the effector cell led to an inhibition of cytotoxicity (22), this was interpreted as due to the dispersion of the GA elements in the killer cell and the loss of polarity of the cytotoxic secretory process.

Comparison with Previous Studies on the Effect of Altered Microtubule Assembly Status on Secretion

The literature on the effect of colchicine- or vinblastine-induced disassembly of microtubules on the secretion of specific secretory components of a variety of cell types is too voluminous for a detailed review in this paper. The results obtained have ranged from no effect of 30 μM colchicine on amylase secretion by isolated mouse pancreas acini (40), to a 70–75% reduction by 10 μM colchicine treatment on the net triglycerides released from perfused mouse livers (24). It is noteworthy, however, that in a number of cases where secretion was partially inhibited by colchicine, it was demonstrated that the addition of terminal sugars to the oligosaccharide

chains of the secretory protein was unchanged, indicating that the transfer of the protein from the RER to the GA was unaffected by microtubule disassembly (1, 5). The partial inhibition of secretion in several of these cases was attributed to an effect on the transfer process from the GA to the cell surface (28, 33–35). It is important to realize that microtubule-dissociating drugs besides their capacity to cause microtubule disassembly, may be membrane interactive compounds (39), and therefore might affect the fusion of GA-derived secretory vesicles with the plasma membrane, independent on the question of microtubule assembly status. On the other hand, there have also been a smaller number of reports that in certain cases colchicine inhibits the transfer of secretory proteins from the RER to the GA (cf. reference 7), but they appear to be in the minority. Taking all of these results into account, we conclude that our findings with the G integral membrane protein are consistent with the majority of the secretory studies (cf. reference 8); that microtubule disassembly has no significant effect on the process of transfer from the RER to the GA and minimal (~50%) or no inhibition of the extent of the transfer process from the GA to the plasma membrane, of either integral membrane or secretory proteins.

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