Microtubule-depolymerizing Agents Inhibit Moloney Murine Leukaemia Virus Production

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SUMMARY

The effects of microtubule-depolymerizing agents on virion production in MJD-54 cells chronically infected with Moloney murine leukaemia virus were examined. By measuring the amount of reverse transcriptase activity remaining in particles recovered from culture fluids, we found that incubation with 1 \( \mu \)M- or 10 \( \mu \)M-colchicine, vinblastine or nocodazole resulted in 30 to 40% decreases in virus production. The decrease in virus production did not seem to be due to general damage to the cells since cellular RNA and protein synthesis were only slightly, if at all, inhibited by the drug treatment (<10%). Furthermore, virus proteins accumulated inside drug-treated cells, viz, \([^{35}S]\)methionine-labelled Pr65\(^{gag}\) showed a 1-5-fold increase over a 3 h continuous label interval. Consistent with this accumulation of virus protein, electron microscope studies showed that inside drug-treated cells there was a 2- to 2.5-fold accumulation of virions within cytoplasmic vesicles. All of these results support the idea that cytoplasmic microtubules play a role in the production of murine leukaemia virus.

INTRODUCTION

Cytoplasmic microtubules have long been implicated in a variety of cell functions such as the maintenance of cell shape, cellular locomotion and chromosome movement (for review, see Dustin, 1978; Stebbings & Hyams, 1979), the regulation of various plasma membrane phenomena (Edelman et al., 1973; Oliver et al., 1980) and the transport of secretory granules (Porter, 1966; Williams, 1975; Sherline et al., 1977). The most convenient way to examine the role of cytoplasmic microtubules has been to see the effects of microtubule-depolymerizing agents on each cellular function. Thus far, there have only been a few instances where the role of cytoplasmic microtubules in virion production from infected cells has been examined. For example, with Semliki Forest virus it was shown that such drugs inhibited virus production (Richardson & Vance, 1978) while, for vesicular stomatitis virus (VSV), production was not affected at all by these agents (Gentry & Bussereau, 1980).

In the case of murine leukaemia virus (MuLV)-infected cells, virus assembly is thought to occur at the plasma membrane (Yeger et al., 1978; Mooren et al., 1980) where virus precursor proteins, e.g. Pr65\(^{gag}\) and RNA are brought to the region underlying the plasma membrane from their sites of synthesis. Assembly occurs by budding of the multimeric Pr65\(^{gag}/RNA\) complexes at the cell surface. However, it is still not known how Pr65\(^{gag}\) is recruited to the plasma membrane. Since microtubules have been implicated in the regulation of the distribution of various plasma membrane proteins (Edelman et al., 1973; Oliver et al., 1980) as well as in the intracellular transport of cellular vacuoles, organelles and granules (Porter, 1966; Williams, 1975; Sherline et al., 1977), we thought it worthwhile to investigate the role played by microtubules in C-type MuLV production.
METHODS

Cells and materials. MJD-54 cells chronically infected with Moloney murine leukaemia virus (M-MuLV) were cultivated in Dulbecco's modified Eagle's minimal essential medium (DMEM, Gibco) supplemented with 10% foetal calf serum (FCS, Flow Laboratories) in a 5% CO₂ incubator at 37 °C. Microtubule-depolymerizing agents used included colchicine (Calbiochem), vinblastine sulphate (a generous gift from Eli Lilly) and nocodazole (Aldrich). Lumicolchicine was made by u.v. irradiation of colchicine by the method of Wilson et al. (1974). [¹⁴C]leucine (270 mCi/mmol) and [³H]uridine (2 to 10 Ci/mmol) were obtained from New England Nuclear. Goat antiserum against Rauscher leukaemia virus p30 antigen was generously provided by the Resources Program of the National Cancer Institute (lot 78S-223).

Assay of cell supernatants for viruses. When the cells reached confluence (1 × 10⁷ to 3 × 10⁷ cells/flask) in 75 cm² T-flasks, fresh medium was added with or without a drug and cells were preincubated for 1 h, then the medium was changed, again with or without a drug and cells were incubated further for 1, 2 or 3 h. Culture fluids were collected and centrifuged at 4000 g for 20 min to remove floating cells or cell debris. The supernatants were then centrifuged at 114000 g for 1 h at 4 °C and the pellets were usually suspended in 0.5 ml of TNE buffer (10 mM-tris-HCl pH 7.5, 100 mM-NaCl and 1 mM-EDTA). Aliquots of samples were assayed using oligo(dT).poly(rA) for reverse transcriptase activity (Yoshinaka et al., 1980). Virions were labelled by incubation with [³H]uridine at 10 µCi/ml media for 3 h and virus suspensions, obtained as above, were then layered on to a linear 10% (w/w) to 50% (w/w) sucrose gradient in TNE buffer. After centrifugation at 80,000 g at 4 °C overnight, the gradient was fractionated. Acid-insoluble material was collected and the radioactivity measured for each fraction as described below.

Measurement of protein and RNA synthesis. Cells were cultivated in 35 mm dishes with DMEM plus 10% FCS. The medium was changed to DMEM for the labelling experiments. Cells were first pretreated with or without a drug for 1 h, [¹⁴C]leucine or [³H]uridine was added to a final concentration of 1 µCi/ml (in the case of [³H]uridine, non-radioactive uridine was added to a final concentration of 0.00125 µg/ml) and the cells were further incubated. After incubation cells were lysed with 0.1% SDS and the 5% trichloroacetic acid (TCA)-insoluble fraction was collected on glass fibre filters. The radioactivity was measured using Liquifluor (New England Nuclear) in a liquid scintillation counter.

Immunoprecipitation of virus proteins. After cells grown in T-flasks were preincubated in DMEM without methionine for 15 min, they were labelled with 20 µCi/ml [³⁵S]methionine for appropriate times in DMEM without methionine plus 5% dialysed FCS. In the case of pulse-chase experiments, cells were fed by addition of DMEM plus 10% FCS during the chase period. At the end of the incubation period, cells were washed once with phosphate-buffered saline (PBS, pH 7.2) and lysed with 5 ml lysing buffer [20 mM-tris–HCl pH 7.5, 50 mM-NaCl, 0.5% Nonidet P40 (NP40), 0.5% sodium deoxycholate, 0.1% SDS and 1 mM-phenylmethylsulphonyl fluoride]. Cell lysates were centrifuged at 39000 g for 1 h at 4 °C and 0.5 ml of supernatant was taken to which 20 µl of anti-p30 serum was added. After incubation for 15 min at 37 °C, cell lysates were maintained overnight at 4 °C. A 200 µl solution of Staphylococcus aureus (100 mg/ml in lysing buffer of protein A lyophilized cell powder, Sigma) was added to cell lysates and the mixture was agitated for 1 h at 4 °C. Immunoprecipitates were washed three times with cold washing buffer (20 mM-tris–HCl pH 7.5 and 50 mM-NaCl) and once with distilled water, all at 1000 g for 20 min. Then SDS–polyacrylamide gel electrophoresis (PAGE) was performed as previously described (Yoshinaka et al., 1980). A 200 µl amount of sample buffer for electrophoresis was added to the immunoprecipitates and they were boiled for 3 min. Then a final centrifugation was performed to separate the immunoprecipitated complex from the bacteria. The
supernatants were then analysed by SDS–PAGE (10% acrylamide) and processed for fluorography.

**Electron microscopy.** Cells were cultured in T-flasks. After treatment with or without a drug they were scraped into PBS using a rubber policeman and centrifuged. The pellets were fixed with 2% glutaraldehyde in 0.1 M-sodium cacodylate buffer pH 7.4. Post-fixation with 1% OsO₄, dehydration, embedding, sectioning and staining with lead citrate were performed as previously described (Luftig *et al.*, 1974).

**RESULTS**

**Effects of microtubule-depolymerizing agents on M-MuLV production**

In these experiments we initially used vinblastine to depolymerize cytoplasmic microtubules; however, similar results were found with colchicine and nocodazole. MJD-54 cells were pre-incubated with or without 10 μM-vinblastine for 1 h. This preincubation period was used in all subsequent experiments since it was found that 60 min was sufficient time for the drugs to depolymerize the microtubule networks in over 90% of the cells, as assayed by indirect immunofluorescence using anti-tubulin serum (data not shown). Then the cells were further incubated in fresh medium for 1, 2 or 3 h in the absence or presence of the drug. At the end of the incubation period, culture fluids were collected and virions were recovered by centrifugation. Differences in the number of virions recovered were assayed for by measuring reverse transcriptase activity. Fig. 1 shows the accumulation of M-MuLV virions in culture fluids during several incubation periods. Compared to non-treated cells which showed a linear accumulation of virions at a rate of \(4.75 \times 10^4\) ct/min \([3H]\)TTP precipitated/h, vinblastine-treated cells produced virions at about 60% of the control rate for each time point examined. For vinblastine-treated cells, the rate of accumulation of virions was \(1.4 \times 10^4\) ct/min \([3H]\)TTP precipitated/h. This decrease in virion yield, as assayed for by measuring reverse transcriptase activity, could also be seen when virions were assayed by measuring the amount of \([3H]\)uridine incorporated into virions recovered after sucrose density gradient centrifugation (Fig. 2). The peak value clearly shows that again there is a 40% decrease in virus production by 3 to 4 h after 10 μM-vinblastine treatment.

In order to show that the decrease seen above was due to the specific effect of microtubule depolymerization and not a secondary effect of vinblastine (DeBrabander & Borgers, 1975), we then examined the effects of two other microtubule-depolymerizing drugs, colchicine and nocodazole, on virus production. As shown in Table 1, after a 3 h incubation with each of the drugs at a concentration of 10 μM, virus production was similarly inhibited, i.e. by 40% and 32% respectively. Thus, on the average for the three drugs we consider that there is about a 40% inhibition by 3 h after treatment. It should also be noted from this table that each drug inhibited virus production to a similar degree (about 30%) even at lower (1 μM) concentrations.

The specificity is also supported by the experiment with lumicolchicine, a derivative of colchicine which has no effect on depolymerizing microtubules but does retain its ability to affect nucleoside transport and intramembrane particle aggregation (Wilson *et al.*, 1974; Furucht & Scott, 1975). We found that at a 1 μM concentration lumicolchicine had no inhibitory effect on M-MuLV production. This is in contrast to the 35% inhibitory effect of 1 μM-colchicine (Table 2).

**Effects on cellular processes of MJD-54 cells after treatment with microtubule-depolymerizing drugs**

**Cell viability**

In all of the experiments we have performed (i.e. using 1 or 10 μM amounts of each drug for
Fig. 1. Effect of vinblastine on the accumulation of Moloney virions in culture fluids. MJD-54 cells were preincubated in the absence or presence of 10 μM-vinblastine for 1 h and further incubated for 1, 2, or 3 h in the absence (---) or presence (----) of the drug. After each incubation period culture fluids were collected and virions were recovered by ultracentrifugation. Virus yield was measured in terms of reverse transcriptase activity, assaying the incorporation of [3H]TTP into a TCA-insoluble fraction. Each point represents the mean value (+3 %) from two flasks.

Fig. 2. Banding of Moloney virions on a sucrose density gradient. Virions labelled with [3H]uridine were recovered as in Fig. 1 from 0 (-----) and 10 μM- (------) vinblastine-treated cells. After the virus suspension was centrifuged on a linear 10 to 50% sucrose gradient, the gradient was fractionated. TCA-insoluble material was collected from each fraction and the radioactivity measured. The data obtained from another set of flasks showed a similar banding pattern (the peak value from non-treated cells was 620 ct/min and the one from vinblastine-treated cells was 330 ct/min).

up to 4 h exposure), cell viability was maintained at a greater than 95 % level as quantified by the trypan blue exclusion assay. Thus, the 40% decrease in M-MuLV production does not represent a decrease due to the death of 40% of the cells.

Cellular macromolecular synthesis

MJD-54 is a chronically infected mouse fibroblast cell line and has multiple copies of the M-MuLV genome integrated into the host genome. Therefore, overall virus production in
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Table 1. Production of M-MuLV (%) after treatment with three mierotubule-depolymerizing drugs*

<table>
<thead>
<tr>
<th>Concentration (μM)</th>
<th>Colchicine</th>
<th>Vinblastine</th>
<th>Nocodazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100 ± 3†</td>
<td>100 ± 6</td>
<td>100 ± 13</td>
</tr>
<tr>
<td>1</td>
<td>71 ± 7</td>
<td>72 ± 6</td>
<td>73 ± 1</td>
</tr>
<tr>
<td>10</td>
<td>60 ± 10</td>
<td>60 ± 3</td>
<td>68 ± 3</td>
</tr>
</tbody>
</table>

* MJD-54 cells were preincubated in the absence or presence of the drug for 1 h and then further incubated for 3 h in fresh medium with or without the drug. Virus was recovered from culture fluids and virus yield was measured in terms of reverse transcriptase activity. Assuming the virus yield given in absolute terms of TCA-precipitable [3H]TTP in non-treated cells as 100%, the yield in drug-treated cells is shown as a percentage.

† The average number of counts corresponding to 100% are 630,900, 343,700 and 173,000 for 0 μM-colchicine, vinblastine and nocodazole respectively. All values represent the average ± standard error for three separate experiments.

Table 2. Effect of lumicolchicine on virus production*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TCA-precipitable [3H]TTP (ct/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41,720</td>
</tr>
<tr>
<td>Lumicolchicine (1 μM)</td>
<td>39,240</td>
</tr>
<tr>
<td>Colchicine (1 μM)</td>
<td>26,660</td>
</tr>
</tbody>
</table>

* The experimental procedure was the same as in Table 1. Each value represents the average from two T-flasks (±6%).

such cells will depend on both total cell protein and RNA synthesis. To determine whether the mierotubule-depolymerizing drugs used might be decreasing virus production by inhibitory effects on total cellular protein and RNA synthesis, we measured incorporation of [14C]leucine and [3H]uridine into total acid-insoluble material after drug treatment. MJD-54 cells were incubated in the presence or absence of 10 μM-colchicine, vinblastine or nocodazole for 1 h up to 4 h. We found that both protein and RNA were synthesized almost normally in cells treated with each drug during the incubation period, although a slight (< 10%) inhibition of synthesis may be occurring (data not shown).

Analysis of the major virus protein Pr65\textsuperscript{ gag}

It has now been shown that MuLV particles are assembled from polyproteins such as Pr65\textsuperscript{ gag} which are subsequently cleaved during or after budding of the immature virion from the cell surface (Yoshinaka & Luftig, 1977a, b; Yeger et al., 1978; Lu \textit{et al.}, 1979; Yoshinaka \textit{et al.}, 1980). We determined if the decrease of virus production observed in drug-treated cells was accompanied by a decrease in the synthesis of precursors such as Pr65\textsuperscript{ gag}. Pr65\textsuperscript{ gag} (Fig. 3a) is the major precursor polyprotein to the virus internal structural proteins p15, p30, p12 and p10. In this experiment, MJD-54 cells were preincubated with or without 10 μM-colchicine for 1 h, then labelled with [35S]methionine for 3 h in the presence or absence of the drug. After the cells were lysed they were immunoprecipitated with p30 antiserum and analysed by SDS–PAGE. We observed that the amount of Pr65\textsuperscript{ gag} inside cells was significantly increased (about 1.5-fold over control) (Fig. 3b) after colchicine treatment (Fig. 3c). The same increase occurred with vinblastine and nocodazole treatment (Fig. 3d, e); a similar (about 1.5-fold) increase over control for p30 was noted. Thus, the 40% inhibition in virus production seen after drug treatment cannot be attributed to an inhibition in virus protein synthesis; in fact, it appears that there is an accumulation of Pr65\textsuperscript{ gag} and p30 in such treated cells.

In order to understand how such an accumulation may arise, the fate of Pr65\textsuperscript{ gag} synthesized in these drug-treated cells was followed by a pulse-chase experiment. Cells were
Fig. 3. Continuous labelling of virus-specific proteins in MJD-54 cells. Cells were preincubated in the presence or absence of the drug for 1 h, labelled with [35S]methionine for 3 h and then lysed. The immunoprecipitates by anti-p30 serum were analysed on 10% SDS-polyacrylamide gels and processed by fluorography. Lane (a) shows a sample of M-MuLV virions; lane (b) non-treated cells; lanes (c to e) are cells treated with 10 μM-colchicine, vinblastine and nocodazole respectively. Each lane represents the immunoprecipitate from one flask. Experiments were performed in duplicate with essentially identical results. Quantification was performed by scanning the autoradiogram. The respective percentages of Pr65<sub>ag</sub> relative to 100% for non-treated cells were found to be 169, 150 and 157% for colchicine, vinblastine and nocodazole-treated cells respectively; for p30 relative to 100% for non-treated cells, the values were found to be 142, 158 and 120% respectively.

preincubated with 10 μM-colchicine for 1 h and then pulse-labelled for 15 min with [35S]methionine and chased in complete medium for 1, 2 or 3 h in the presence of colchicine. A control was done in parallel where all steps were done in the absence of colchicine. Then the cell lysates were immunoprecipitated with p30 antiserum and analysed by SDS-PAGE. It can be seen that for the untreated cells (Fig. 4) the Pr65<sub>ag</sub> pulse-label rapidly disappeared during the chase period, with a 70% and a 95% decrease in label by 1 and 3 h post-chase respectively. This decrease of labelled intracellular Pr65<sub>ag</sub> parallels the results obtained by Schultz et al. (1979) and reflects the assembly of uncleaved Pr65<sub>ag</sub> into budding virions. In the colchicine-treated cells, Pr65<sub>ag</sub> pulse-label also disappeared during the chase period. However, the uncleaved Pr65<sub>ag</sub> remained longer inside the cells with only a 60% and a 75% decrease occurring by 1 and 3 h post-chase respectively. This observation is consistent with our finding that virus release into the culture medium is decreased by 40% in the presence of colchicine (Table 1). Also, p30 remained longer inside colchicine-treated cells during the chase. It should be noted, however, that the virions released from colchicine-treated cells show no difference in the ratio of [35S]methionine-labelled Pr65<sub>ag</sub> to p30 from the virions of...
Fig. 4. Pulse-chase labelling of viral Pr65\textsuperscript{agg} in MJD-54 cells. Cells were preincubated in the absence or presence of 10 µM-colchicine for 1 h and then pulse-labelled with \[^{35}\text{S}\]methionine for 15 min. For the chase, cells were further incubated in complete medium with or without the drug. Lane (a) pulse; lanes (b to d) show control chase at (b) 1 h, (c) 2 h, (d) 3 h; lane (e) pulse; lanes (f to h) show colchicine chase at (f) 1 h, (g) 2 h, (h) 3 h. At the end of the chase cells were lysed, immunoprecipitated with anti-p30 serum and analysed as in Fig. 3. Assuming a value of 100% for Pr65\textsuperscript{agg} from the pulse lanes, the percentages of Pr65\textsuperscript{agg} remaining during the chase period (1, 2 or 3 h post-chase) were calculated for non-treated (30, 16 and 6%) and colchicine-treated cells (43, 33 and 24%) respectively.

Table 3. \textit{Quantification of virus particles seen in thin-section electron micrographs*}

<table>
<thead>
<tr>
<th>Vinblastine (µM)</th>
<th>Average no./cell/section</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Intracellular virions</td>
</tr>
<tr>
<td>0</td>
<td>4.70</td>
</tr>
<tr>
<td>1</td>
<td>7.59</td>
</tr>
<tr>
<td>10</td>
<td>11.10</td>
</tr>
</tbody>
</table>

* Virus particles were counted per nucleated cell profile. At least 100 different cell profiles were counted for each average value. The standard error varied from 5 to 12% over the different data points. When surface buds were examined, on average there was about 0.4 per nucleated cell profile at each concentration; however, the standard error was over 50% so that the reliability of such data points is questionable. When an experiment similar to the above was performed, where 10 µM-colchicine had been added for 0, 2 and 4 h, we found the average number of intracellular virions was 4.14, 6.46 and 7.57 respectively. There was also about the same number of vesicles per nucleated cell profile, viz, on average 4.5 ± 0.6 for each point.

† Drug treatment was for 3 h.

‡ This column includes cytoplasmic structures where a complete or partial membrane outline can be seen [Fig. 5, vesicles (vs) as well as clusters (cl)] or virus in which a membrane image is not clearly visualized. We considered these latter images to also be vesicles because, when sections are cut through cells that have spontaneously lysed during the preparation, membranes are clearly seen on all vesicles containing virus. It may be that, due to the angle at which the section was cut and/or to fixation and staining problems, the intracellular membrane outline is only suggested by the centrally gathered cluster of virus particles.
Fig. 5. Electron micrographs of sectioned MJD-54 cells: (a) non-treated, (b) treated with 10 μM-vinblastine and (c) treated with 10 μM-colchicine. Representative cytoplasmic structures, vesicles (vs) and clusters (cl), are seen with virions (arrows) inside them; O in (c) is outside of the cell. All bar markers represent 0.1 μM.
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non-treated cells (M. Satake & R. B. Luftig, unpublished results). If colchicine had also inhibited the cleavage of Pr65<sup>agg</sup> to p30, we would have expected some increase of Pr65<sup>agg</sup> relative to p30 in these virions. Thus, colchicine treatment apparently delays but does not inhibit the cleavage of Pr65<sup>agg</sup>.

Electron microscopic examination of drug-treated MJD-54 cells

Previously, we have reported that budding can take place in cytoplasmic vesicles (Luftig et al., 1974) as well as at the plasma membrane. Budding into cytoplasmic vesicles has also been seen with other enveloped viruses, e.g. in Semliki Forest virus (Grimley et al., 1968) and VSV-infected chick embryo fibroblasts (Johnson & Schlesinger, 1980). The results of our immunoprecipitation study showed that there was an accumulation of Pr65<sup>agg</sup> and p30 inside drug-treated cells. To examine the possibility that virus particles may accumulate inside cytoplasmic vesicles of such cells, cells were fixed, sectioned and examined by electron microscopy. We found that there was a 2- to 2.5-fold overall increase of intracellular virions in both vinblastine- and colchicine-treated cells (Table 3; Fig. 5). Since the particles were clustered in the same number of vesicles (see footnotes to Table 3), this means in fact that on average there are about 2.5 times as many particles in vesicles of drug-treated (Fig. 5 b, c) as in non-drug-treated cells (Fig. 5a).

DISCUSSION

Three different microtubule-depolymerizing drugs (colchicine, vinblastine and nocodazole), when added to MJD-54 (M-MuLV) cells for 3 h at 10 μM, were all found to inhibit M-MuLV production by about 40%. This inhibition of virus production appears due to a specific action of the drugs on cytoplasmic microtubules since: (i) cytoplasmic microtubule networks disappeared completely when cells were treated with the drugs; (ii) all three drugs decreased virus production to almost the same extent at both 1 and 10 μM; (iii) the inhibition of virus production was observed over a short incubation period (1 to 3 h) with a low concentration (1 and 10 μM) of each drug; and (iv) lumicolchicine, an inert analogue of colchicine, had no inhibitory effect on virus production.

The possibility that the inhibition of virus production was due to the depressed synthesis of cellular macromolecules was excluded since cellular protein and RNA were synthesized normally in drug-treated as well as in non-treated cells. Furthermore, virus-specific Pr65<sup>agg</sup> protein synthesis is not inhibited. Instead, continuous labelling for 3 h shows that Pr65<sup>agg</sup> is accumulated inside drug-treated cells. This observation that the drug treatment has slowed down virus production is also in agreement with the finding that Pr65<sup>agg</sup>, once synthesized, remains longer in drug-treated cells than in control cells. Some of these precursors can apparently still assemble into virions on cytoplasmic vesicles since electron microscope studies showed that there were increased numbers of virus particles inside drug-treated cells.

The question posed by the above findings is how microtubules play a role in M-MuLV production at the subcellular level. Some light has been cast on this question by observations using cytoskeletal preparations of MJD-54 cells, which were prepared by extraction of cells with a low (0.1%) concentration of non-ionic detergent (NP40) under conditions of microtubule stabilization (Solomon et al., 1979). The preparations were fixed and examined for p30 antigen by indirect immunofluorescence. We observed that the cytoskeletons of non-drug-treated cells showed a significant amount of p30-associated fluorescence which remained even after detergent extraction with NP40. In contrast, this p30 fluorescence was found to be greatly diminished in cytoskeletons from vinblastine-pretreated cells (M. Satake & R. B. Luftig, unpublished results). Thus, these observations, when taken into consideration together with the notion that cytoskeletons serve as a potential site for synthesis and assembly of poliovirus- and VSV-specific proteins inside infected cells (Lenk & Penman,
1979; Cervera et al., 1981), strongly imply that cytoplasmic microtubules may play a partial role in efficiently recruiting MuLV Pr65\textsuperscript{gag} from its site of synthesis to the plasma membrane where budding will take place. However, we recognize that cytoskeletons are complicated structures composed of microfilaments, intermediate filaments and other structural proteins as well as microtubules (Ben Ze'ev et al., 1979; Lenk & Penman, 1979; Solomon et al., 1979; Cervera et al., 1981). Thus, a more detailed examination of our initial findings might reveal a non-microtubule-mediated recruitment of Pr65\textsuperscript{gag} to the plasma membrane. For example, microfilaments have already been implicated in the transport of poxvirus inside infected cells (Hiller et al., 1979) as well as in the final stage of release of assembled influenza virus particles (Griffin & Compans, 1979).

Finally, we would like to contrast our studies with those of others who have examined the involvement of cytoplasmic microtubules in enveloped virus production. For Semliki Forest virus-infected cells, it was shown that virus production was inhibited by 75 to 90% using both colchicine and nocodazole (Richardson & Vance, 1978). In these studies it should be noted that a relatively high (100 \( \mu \text{M} \)) concentration of each drug was used. We have found that at a 1 or 10 \( \mu \text{M} \) concentration of each drug there was a 30 to 40% level of inhibition in MuLV production. For VSV-infected mouse 3T3 cells it was shown that concentrations of colchicine as high as 100 \( \mu \text{M} \) did not inhibit virus production (Gentry & Bussereau, 1980). Thus, the depolymerization of microtubules appears to differentially affect enveloped RNA virus production, ranging from a complete block to no inhibition.

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