Effect of Interferon on the Replication of Mink Cell Focus-Inducing Virus in Murine Cells: Synthesis, Processing, Assembly, and Release of Viral Proteins

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Treatment of mink cell focus-inducing (MCF) virus (isolate AK-13) producing SC-1 cells with mouse fibroblast interferon (150 to 600 U/ml) led to a 100-fold decrease in the release of infectious virus, whereas there was a 2.5- to 10-fold decrease in various parameters of virus particle release. Analysis of labeled virion proteins indicated that a temporal change in virion protein composition occurred after interferon treatment. After a 24-h exposure of chronically infected cells to interferon, the virions produced contained a 85,000-dalton glycoprotein (apparently of nonviral origin) which was in excess of the virus envelope glycoprotein gp70. Particles produced from cells treated with interferon for 32 to 48 h were nearly devoid of gp70 and contained substantially lower quantities of p30. Intracellular processing of viral precursor polyproteins to the mature virion structural proteins was not altered in the presence of interferon. However, an accumulation of the viral p30 and p12E proteins was observed in interferon-treated cells, consistent with an increase in cell-associated virions. Immunoprecipitation analysis of the tissue culture fluids from [³⁵S]methionine-labeled control and interferon-treated cells revealed a marked decrease in p30 and p15E/p12E released after interferon treatment. In contrast, gp70 did not accumulate in interferon-treated cells, but was released into the culture medium in a form that was neither pelletable nor associated with p15E/p12E.

Interferons are a family of cellular glycoproteins synthesized in response to viral infection or nonviral inducers such as double-stranded polyribonucleotides and certain halogenated pyrimidols (for review, see reference 16). Exposure of cells to interferon results in the establishment of an "antiviral state" as manifested by the inhibition of the replication of a wide variety of DNA and RNA viruses. Although the mechanism by which interferon inhibits viral replication is not fully defined, the majority of viruses are inhibited at the level of mRNA translation (16, 21, 22, 28). However, our own laboratory and others have demonstrated that interferon inhibits the replication of murine retroviruses in both chronically and acutely infected cells, and this inhibition occurs after the synthesis of viral RNA and proteins (4, 15, 32-35). A number of studies have suggested that interferon interferes with retrovirus assembly and maturation, leading to the "trapping" of the virions at the cell surface (5, 10, 34, 36). In systems where virions were released from interferon-treated cells, the released particles had reduced infectivity. Examination of the structural proteins of virions released from interferon-treated cells had indi-

cated that new proteins, presumably incorrectly processed viral precursor polyproteins, were incorporated into virions (9, 33).

Our previous results had indicated an alteration in assembly as the primary defect in retrovirus production after interferon treatment of both chronically and de novo-infected cells (32, 33, 36). Retrovirus assembly is a complicated process requiring the proper temporal and spatial cleavage of three major precursor proteins: the *gag* precursor (Pr67^{gag}) is cleaved to the virion core proteins (p10, p12, p15, and p30), *gag-pol* (Pr180^{gag-pol}) is cleaved to the virion RNA-dependent DNA polymerase (and presumably *gag* gene products), and the *env* precursor (gPr85^{env}) gives rise to the virion envelope glycoprotein gp70 and the virion matrix protein p15E/p12E (for review, see reference 40). Viral polyproteins function directly in the assembly process; for example, Pr67^{gag} contains both RNA-binding proteins which bind the genomic RNA and proteins which specifically interact with the virion transmembrane protein p15E, affecting attachment of the viral core to the membrane. The *env* precursor gPr85^{env} must be further glycosylated and in a proper configura-

tion in the membrane to permit both the interaction of p15E with Pr67^{gag} and the formation of a stable disulfide linkage between p15E and gp70 before proteolytic cleavage (7, 29, 31).

We have attempted to compare the synthesis, processing, glycosylation, and release of viral proteins in the presence and absence of interferon in order to determine whether interferon-induced alterations in these processes are responsible for the defective assembly. Our studies have concentrated on gp70 expression, since the envelope glycoprotein determines virus infectivity, neutralization, interference, and host range properties (reviewed in reference 40). Additionally, the malignancy of murine lymphosarcoma cell lines appears to correlate with the cell surface display of gp70 (37). Interferon-sensitive SC-1 cells infected with the envelope gene recombinant mink cell focus-inducing (MCF) virus isolated from AKR mice (19) were used in this study. This virus-cell system has several unique properties relevant for studies of the effect of interferon on retrovirus replication. MCF viruses can be titrated by focus assay on mink cells which are insensitive to mouse interferon; thus, the infectivity assay is not affected by the possible presence of residual mouse interferon in the system. Since earlier studies had indicated that interferon may affect viral precursor protein processing, the use of the SC-1/MCF cell line with a substantially slower rate of *env* gene processing (12) could amplify minor interferon-induced differences in this pathway. Lastly, the *in vivo* administration of interferon has been shown to prolong the survival of AKR mice (18). Since this MCF virus is highly leukemogenic and implicated in the appearance of spontaneous T-cell lymphomas in AKR mice (19), information gathered from this *in vitro* study may be relevant to the previously observed antitumor effect of interferon in this mouse strain.

MATERIALS AND METHODS

Cells and viruses. Interferon-sensitive SC-1 cells chronically infected with the AK-13 isolate of AKR-MCF virus (19) were used. Virus was titered by enumerating cytopathic foci on mink lung cells (19).

Interferon. Experiments were performed with a single lot of purified mouse interferon induced in L cells with Newcastle disease virus (specific activity, 1.1×10^7 reference units per mg of protein). The interferon was assayed in L929 cells by the cytopathic assay of Finter (13), using vesicular stomatitis virus as the challenge virus. Interferon titers are expressed in international reference units; 1 U of the international standard titrated as 1 U in our assay. Subconfluent monolayers of chronically infected SC-1 cells were treated, 24 h after trypsinization and passage, with the indicated amount of interferon for the indicated length of time.

Reverse transcriptase assay. The virus was pelleted

from the supernatant fluid at $100,000 \times g$ in an SW40 rotor for 90 min, and the virion-associated reverse transcriptase was assayed as described previously (34).

Electron microscopy. We prepared monolayers for transmission electron microscopy as described previously (36). Thin sections were examined in a Hitachi HU-11E microscope with an accelerating voltage of 75 kV. Pellets of the fixed monolayers were divided into multiple fractions, and sections from the separate fractions were used for counting to minimize sampling error and avoid repetitive counting of a few cells.

Radiolabeling. In experiments where metabolically radiolabeled virus or virion proteins were to be characterized, cells were labeled for a 16-h interval either 8 or 32 h after interferon treatment (24- or 48-h virions, respectively). Labeling with [³⁵S]methionine was with 50 μ Ci/ml in medium containing 5% of the normal level of methionine. Tritiated monosaccharides (Amersham Corp., Arlington Heights, Ill.) were used at 20 μ Ci/ml in a medium containing 10% the normal concentration of glucose.

Pulse-chase experiments were performed at 24 or 48 h after interferon treatment. Cells were preincubated for 1 h in medium lacking methionine before labeling for 30 min with 125 μ Ci of [³⁵S]methionine per ml (Amersham; specific activity, 1,100 to 1,300 Ci/mmol). Chase was in complete medium containing a 100-fold excess of unlabeled L-methionine. Cells were washed twice with phosphate-buffered saline before preparation of cell extracts.

We performed lactoperoxidase radioiodination of cell surface proteins after washing the monolayers twice with buffer (phosphate-buffered saline [pH 7.4] or 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES; pH 7.4]-buffered saline as indicated). Cells were labeled at room temperature for 10 min in 1 ml of the indicated saline solution containing 5 μ g of lactoperoxidase (Calbiochem-Behring, B grade) 0.5 mCi of ¹²⁵I, and 10 μ l of 0.03% H₂O₂. If no chase was desired, monolayers were washed and lysed in extraction buffer. Chase was performed after the reaction was stopped by addition of cold wash buffer containing 0.5 mM tyrosine. Cells were washed twice and transferred to fresh medium. After the indicated length of chase, the cells were washed and supernatants and cells were analyzed as described above.

Immunoprecipitation analysis of labeled proteins. Preparation of cell extracts and immunoprecipitation with monospecific goat antisera to purified retrovirus proteins was described previously (3). To compensate for differences in protein synthesis, each immunoprecipitation was done with approximately 10^8 dpm of cell extract; the volume of each sample was adjusted to 1 ml with extraction buffer. Analysis of viral proteins released into the medium was done with 1.0 ml of clarified supernatant (4,000 $\times g$, 5 min) adjusted to 1% Triton X-100 and 0.5% deoxycholate. Cell extracts and supernatants were precipitated with normal goat serum before specific immunoprecipitation with a previously determined amount of antiserum. *Staphylococcus aureus* immunoabsorbant was used to collect antigen-antibody complexes by centrifugation. Pellets were washed twice with 20 mM Tris (pH 7.4), 100 mM NaCl, 1 mM EDTA, and 0.5% Nonidet P-40 and once with the same mixture containing 2.5 M KCl.

Particles were analyzed after centrifugation through

a 20% sucrose cushion. Seven milliliters of radiolabeled tissue culture supernatant was layered over 5 ml of 20% sucrose in Tris-buffered saline (TBS; 20 mM Tris, pH 7.4, and 100 mM NaCl) and centrifuged for 90 min at 35,000 rpm in an SW41 rotor at 4°C. Tubes were fractionated from the top, and the upper 4 ml was used as a post-centrifugation sample. Just before removal of the sucrose layer, the sides of the tubes were washed three times with TBS. At a point where 1 ml of sucrose remained above the pellet, the sides of the tubes were again washed three times with TBS. The remaining sucrose was removed, and the pellet was gently washed twice with TBS. Pellets were suspended in the extraction buffer used for immunoprecipitation and analyzed either directly on polyacrylamide gels or after immunoprecipitation with specific antisera. Analysis of virus prepared by banding on sucrose gradients gave identical results.

Polyacrylamide gel electrophoresis and quantitation of labeled protein bands. Samples were analyzed on 12.5 or 15% sodium dodecyl sulfate-polyacrylamide slab gels, using the discontinuous system described by Laemmli (24). Fluorography was performed according to the procedure of Bonner and Laskey (8), and the dried gels were exposed to preflashed Chronex 4 X-ray film at -70°C.

Autoradiographs of the dried gels were quantitated by the use of an integrating spectrophotometer (Ortec model 4310) when an appropriate exposure time was available in which the film gave a linear response. Routinely, labeled bands were excised from the dried gels, dissolved in 0.3 ml of 15% hydrogen peroxide, and counted in Aquasol II liquid scintillation fluid (New England Nuclear Corp.). The two methods gave comparable results.

RESULTS

Interferon treatment of MCF virus-producing SC-1 cells leads to the production of noninfectious virus. SC-1 cells chronically infected with the AK-13 isolate of MCF virus were treated with 150 to 600 U of purified mouse fibroblast interferon for 24 or 48 h. The levels of infectious virus present in the medium of interferon-treated cultures were reduced by 100-fold, but reverse transcriptase assay of the same supernatants indicated only a 10-fold reduction in virus particles. Metabolic labeling of virions with [³⁵S]methionine indicated a 2.5- to 10-fold reduction in virus particles (Table 1). These results are consistent with earlier studies with AKR-L1 and Rauscher virus-infected cells which had indicated that noninfectious virus particles were formed in the presence of interferon.

Analysis of the number of cell-associated virus particles produced by interferon-treated SC-1/MCF cells was performed by electron microscopy. Transmission electron microscopy (TEM) of representative sections of SC-1/MCF cells indicated that the block in virus release occurred at a step after budding. The number of plasma membrane-associated virus particles visible by TEM of thin sections was increased by approxi-

TABLE 1. Effect of interferon on MCF virus release

Expt no.	Interferon treatment	Infectious virus (FFU/ml)	Reverse transcriptase activity (%)	Plasma membrane-associated virions	[³⁵ S]methionine-labeled virions
1	None 300 U/ml, 48 h	4×10^4 3×10^2	100 ^a 11	1,983 ^b 4,894	
2	None 600 U/ml, 24 h		100 ^c 23		194,660 ^d 42,310
	300 U/ml, 48 h		14		31,453
	600 U/ml, 48 h		15		29,694

^a 100% = 120 pmol of [³H]dTTP incorporated into DNA in response to poly(rA·dT)₁₂₋₁₈ per 20 μl of pelleted virus obtained from 40 ml of culture fluid.

^b TEM, 600 cells counted.

^c 100% = 223,000 cpm incorporated.

^d Total hot trichloroacetic acid-precipitable cpm in samples of [³⁵S]methionine-labeled virus particles prepared as described in Materials and Methods.

mately 2.5-fold in interferon-treated cells when compared with untreated SC-1/MCF cells (Table 1). We have previously shown that the estimates of virus particles on the cell surface quantitated by scanning electron microscopy and computer-generated image analysis are in complete agreement with TEM estimates (36). Ultrastructurally, there was no detectable difference between virions assembled in the presence and absence of interferon.

Viral protein synthesis and processing in control and interferon-treated cells. Viral protein synthesis and processing were studied by pulse-chase labeling and immunoprecipitation analysis. Since other authors (27) have indicated that interferon may exert its inhibitory effect on membrane budding viruses by virtue of a tunicamycin-like activity associated with or induced by interferon, tunicamycin-treated cells were also included for comparison. Figure 1 shows the synthesis and processing of gp70 in SC-1 cells infected with MCF virus as well as in infected cells treated with interferon or tunicamycin. Unlike tunicamycin treatment, which leads to the accumulation of a nonglycosylated precursor to the virion envelope glycoprotein (Pr69^{env}), treatment with interferon did not lead to an inhibition of glycosylation or processing. The major difference observed in the synthesis and processing of the envelope glycoprotein precursor related to the accumulation of the nonglycosylated virion matrix protein p15E/

FIG. 1. Anti-gp70 immunoprecipitates of cell extracts from SC-1/MCF cells treated with interferon or tunicamycin. SC-1/MCF cells were treated with 600 U of purified mouse interferon per ml for 48 h before a 30-min pulse-labeling with [35 S]methionine as described in the text. Parallel control cultures were either labeled at the same time or treated for 1 h before labeling with 1 μ g of tunicamycin per ml. Cell extracts were prepared either at the end of the pulse or after a 90-min chase in medium containing a 100-fold excess of unlabeled L-methionine. Immunoprecipitation with anti-gp70 was as described in the text.

p12E in interferon-treated cells. When cell extracts were immunoprecipitated with antiserum to the virion p15E protein (Fig. 2), there was little difference in the amount p15E and a marked increase in p12E in interferon-treated cells, whereas in control cells only p15E was detected. Previous studies (23; Bilello, unpublished data) as well as those described below indicated that p15E is primarily cell associated and p12E is localized in virions, where it is the major p15E-related protein. It was recently shown that p15E is a precursor of p12E and that a peptide from the carboxy terminus of p15E is removed during its processing to p12E (17).

Analysis of the virion p30-related proteins indicated no major differences in the cleavage pattern of Pr67^{gag} (Fig. 3). In interferon-treated cells, a small but reproducible accumulation of p30 (three- to fivefold increase) was measured by quantitation of the autoradiograph or direct determination of label in the p30 band. The increase in cell-associated p30 is accentuated in

the immunoprecipitation shown, where the same number of counts was used for each precipitation. The observed lack of effect of tunicamycin on processing of Pr67^{gag} and release of p30 into the supernatants of tunicamycin-treated SC-1 cells infected with MCF virus correlated with the results found in other systems (Bilello, Warnecke, Koch, and Ostertag, submitted for publication). Chase of the reverse transcriptase precursor, Pr180^{gag-pol}, also appeared to proceed normally in interferon-treated cells. The $\sim$ 195,000-molecular-weight protein which ran above Pr180^{gag-pol} and was found in anti-gp70, -p30, and -p15E immunoprecipitates was an SC-1 cytoskeletal protein which contaminated immunoprecipitates made from interferon-treated cells. Both the characterization and significance of the interferon-induced increase in the 195-kilodalton protein are under investigation.

Release of viral proteins from control and interferon-treated cells. Our data indicated that the effect of interferon on MCF viral protein synthesis and processing was minimal. However, an intracellular accumulation of p30 and p15E/p12E was observed, whereas no increase in the intracellular level of gp70 occurred. Since other studies have indicated that gp70 can be released

FIG. 2. Anti-p15E immunoprecipitates of cell extracts from SC-1/MCF cells treated with interferon or tunicamycin. Anti-p15E immunoprecipitates were prepared as described in the legend to Fig. 1 and analyzed on a 15% acrylamide-0.2% bisacrylamide slab gel to enhance the separation between p15E and p12E.

FIG. 3. Anti-p30 immunoprecipitates of cell extracts from SC-1/MCF cell treated with interferon or tunicamycin. Anti-p30 immunoprecipitates were prepared from extracts of control, tunicamycin-treated, and interferon-treated cells as described in the legend to Fig. 1.

either free or associated with virus particles (6; Bilello, unpublished data), we decided to examine the effect of interferon on the release of viral proteins as well as the incorporation of these proteins into virus particles. Control and inter-

feron-treated cells (600 U/ml; 48 h) were labeled for 30 min with [³⁵S]methionine and then chased for 2, 4, and 24 h in medium containing a 100-fold excess of unlabeled L-methionine. Analysis of gp70 in the tissue culture fluid at various time points during the 24-h chase indicated that there was no difference in the release of labeled gp70 into the medium in the presence and absence of interferon (Fig. 4a). Similar analysis of p30 release indicated a marked decrease (73 to 87%) in the release of p30 into the supernatant (Fig. 4b) of interferon-treated cells.

Figure 5 shows an immunoprecipitation analysis of tissue culture fluids from cells labeled with [³⁵S]methionine before and after centrifugation to remove virus particles. In lanes A through D, equal volumes (1 ml) of labeled tissue culture fluid were immunoprecipitated with either anti-p30 (panel a) or anti-gp70 (panel b) serum. A comparison of the levels of p30 present in the supernatant from untreated MCF virus-producing SC-1 cells before (lane A) and after (lane C) centrifugation indicated that nearly all (88 to 94%) of the p30 in the supernatant was associated with particles. Tissue culture fluids from interferon-treated SC-1/MCF cells contained very little p30, and nearly all of it appeared to be sedimentable. Similar analysis using anti-gp70 serum indicated that although gp70 was present in the supernatants of both control and interferon-treated cells, a reduction (38 to 43%) in the amount of gp70 present after pelleting of the virus was observed only in supernatants from untreated cells (Fig. 5b).

FIG. 4. Immunoprecipitation of supernatant fluids from [³⁵S]methionine-pulse-labeled control and interferon-treated cells at various times after transfer to medium containing a 100-fold excess of unlabeled L-methionine. (a) Anti-gp70 immunoprecipitates; (b) anti-p30 immunoprecipitates.

FIG. 5. (a) Anti-p30 immunoprecipitates of [^{35}S]methionine-labeled cell-free tissue culture fluids from MCF virus-infected SC-1 cells. A, Supernatant from untreated cells; B, supernatant from cells treated with 600 U of interferon per ml for 48 h; C, same supernatant fluid as in lane A, but centrifuged at $200,000 \times g$ for 90 min over a 20% sucrose cushion to remove virus particles; D, same as lane B, but centrifuged as described above. (b) Anti-gp70 immunoprecipitates.

Analysis of virion structural proteins was performed on particles released from control and interferon-treated cells labeled for 16 h with either [^{35}S]methionine or tritiated monosaccharides. A study of virions produced early after interferon treatment revealed that the reduction in p30 release described above was only marginal in virions labeled between 8 and 24 h after interferon treatment (data not shown). However, the amount of virion-associated gp70 was substantially lower, and the virions contained a new glycoprotein of approximately 85,000 daltons which was the major virion glycoprotein labeled with [^3H]glucosamine (Fig. 6a). The 85-kilodalton glycoprotein could be immunoprecipitated with antiserum prepared against total virus particles, but not with antisera to purified viral proteins (33; Fig. 6 and Bilello, unpublished data), and represented a cellular glycoprotein present in control virions as a minor species.

Figure 6b shows the protein composition of [^{35}S]methionine-labeled virions harvested after 48 h of treatment with interferon, when the inhibitory effect on virus infectivity and particle production was maximal. Lane A shows MCF virions released from untreated SC-1 cells. The virion structural protein, p30, was the major protein component, whereas p15E and p12E were also present in a ratio consistent with that found in other virus preparations. gp70 is visible in the middle of a group of non-virion-specific proteins. The identity of the labeled bands was

confirmed by immunoprecipitations of the virion preparations with antisera to the purified proteins p30 (lane C) and gp70 (lane E). When particles from interferon-treated cells were analyzed, a marked difference in the virion composition was found. Very little p30 or lower-molecular-weight virion proteins were sedimentable from the supernatants from interferon-treated cells (Fig. 6b, lanes B and D). Immunoprecipitation of MCF particles from untreated virus-infected SC-1 cells with anti-gp70 sera precipitated gp70 as well as the virion matrix protein p12E (and a small amount of unprocessed p15E) (Fig. 6b, lane E). In contrast, only a small amount of gp70 and no p12E was immunoprecipitated from particle preparations from interferon-treated MCF-infected SC-1 cells (lane F). Analysis of virion phosphoproteins labeled with ^{32}P , failed to indicate any alteration of the ^{32}P -labeled species (Pr67^{gag}, p12, and other minor species) present in the virion; however, the approximate level of ^{32}P -labeled p12 protein was reduced fivefold in MCF virions released from interferon-treated SC-1 cells (data not shown).

Interferon-mediated inhibition of murine leukemia virus release persists as long as interferon is present in the culture medium, and virus production resumes within 16 to 24 h after interferon removal (34). A reversal experiment was performed as follows. Cells were treated with interferon for 48 h and then washed and refed with medium without interferon. At 24 h after removal of interferon, the cells were labeled for 16 h with [^{35}S]methionine, and the labeled supernatants were analyzed for virion proteins. Supernatant fluids from parallel cultures labeled between 32 and 48 h after interferon treatment were also immunoprecipitated to indicate the extent of the block in retrovirus release before reversal. Interferon treatment resulted in a marked decrease in p15E/p12E release into the supernatant as well as a lack of association of gp70 with either p12E or p15E (Fig. 7). Transfer of the interferon-treated cells to medium without interferon resulted in the increased release of p30 (data not shown) and the association of gp70 with p15E and p12E in immunoprecipitates of supernatants (Fig. 7) or virions (data not shown).

Glycosylation of gp70 after interferon treatment of AKR/MCF-infected SC-1 cells. One possible mechanism for inhibition of the infectivity of membrane budding viruses was the proposed tunicamycin-like activity induced by interferon (26). Although the experiments described above indicated that gp70 was glycosylated, processed, and shed, we were interested to ascertain whether there were any detectable alterations in the carbohydrate moiety of gp70 which would lead to its inability to be assembled into virions.

FIG. 6. (a) Glycoprotein composition of virus particles produced by control and interferon-treated cells. SC-1/MCF cells were labeled for 16 h with tritiated glucosamine and pelleted through a 20% sucrose cushion as described in the text. The first two lanes show the glycoprotein composition of particles produced by control and interferon-treated cells. The last two lanes indicate anti-gp70 immunoprecipitates from the two particle preparations. (b) [35 S]methionine-labeled particles released from control cells and cells treated for 48 h with 600 U of interferon per ml. (A) Control particles; (B) particles from interferon-treated cells; (C) anti-p30 precipitation of A; (D) anti-p30 precipitation of B; (E) anti-gp70 precipitation of A; (F) anti-gp70 precipitation of B.

When gp70 labeled with [3 H]glucosamine, [3 H]mannose, or [3 H]fucose was analyzed in virions or in tissue culture fluids before and after centrifugation, a pattern similar to that observed for [35 S]methionine-labeled gp70 was found (data not shown). Although we observed minor differences in labeling with monosaccharides, we found no evidence for an overt alteration in the glycosylation of free or virus-associated gp70. Differences in the amount of carbohydrate-labeled MCF virus gp70 released by control and interferon-treated SC-1/MCF cells was generally proportional to the extent of overall inhibition of protein synthesis seen in interferon-treated cells (30 to 50% of control).

Expression of gp70 on the surface of interferon-treated SC-1/MCF cells. Our studies of metabolically labeled viral proteins did not exclude the possibility that interferon treatment leads to a redistribution of viral glycoproteins in the cell membrane which may lead to inefficient assembly. It was previously reported that unlike in other retrovirus systems, cell surface iodination of MCF-infected SC-1 cells leads to the detection of both labeled gp70 and gPr85^{env} after immunoprecipitation (12). We performed similar experiments to determine whether we could determine an interferon-induced alteration in the labeling of gp70, its precursor, or both. Our results indicated that detection of gPr85^{env} on

the cell surface appeared to be primarily due to the low viability of the cells in phosphate-buffered saline. Labeling in HEPES-buffered saline, which maintains ~100% cell viability and a minimum of cell breakage (as indicated by a failure to label the intracellular protein actin), resulted in labeling of gp70 exclusively. Under these conditions, no difference was observed in the accessibility of gp70 to lactoperoxidase iodination between control and interferon-treated cells (data not shown).

The turnover of gp70 on the surface of control and interferon-treated cells was examined by following the fate of iodinated gp70 at various times of chase. Control and interferon-treated cells were iodinated and chased for 0.5 or 3 h. The amount of labeled gp70 associated with the cell or present in virions was then determined by immunoprecipitation as described above. No difference was observed in the levels of gp70 associated with control and interferon-treated cells. However, the amount of 125 I-labeled gp70 present in virions released at 3 h after labeling was significantly lower in interferon-treated cultures (Fig. 8), which correlates with our results with metabolic labeling.

DISCUSSION

The results presented here indicate that interferon treatment of SC-1 cells chronically infect-

FIG. 7. Effect of the transfer of interferon-treated cells to normal medium on the release of viral proteins into the tissue culture medium. Anti-p15E immunoprecipitates of [35 S]methionine-labeled virion proteins in the supernatants of control, interferon-treated, and interferon-treated cells shifted for 24 h to normal growth medium for 24 h before labeling. (A) Supernatants from control cells labeled with [35 S]methionine from 32 to 48 h as detailed in the text. (B) Supernatants from SC-1/MCF cells labeled between 32 and 48 h after treatment with 600 U of interferon per ml. (C) Supernatants from a culture of untreated cells grown in parallel to those described in A, but grown for an additional 24 h in fresh normal growth medium before a 16-h labeling with [35 S]methionine. (D) Supernatants from an interferon-treated culture grown for 48 h in parallel to the interferon-treated cells analyzed in lane B, but transferred to fresh medium without interferon for 24 h before a 16-h labeling in the absence of interferon.

ed with MCF virus leads to the formation of noninfectious virions which are deficient in gp70. No evidence was obtained for the inhibition of viral protein synthesis or processing. Similarly, we could not detect a preferential or increased incorporation of uncleaved virion precursor proteins into virions released from interferon-treated cells. Intracellular levels of the

fully processed p30 or p15E/p12E proteins were elevated in interferon-treated cells. Precipitation of the cell-free supernatants from interferon-treated cells with anti-p15E sera failed to detect significant levels of either p15E or p12E (Fig. 7). Although the amount of p30 released from interferon-treated cells was markedly reduced, nearly all of the p30 released from control or interferon-treated cells was in a particulate form. The reduction in the release of p30 and p15E/p12E proteins from interferon-treated cells was accompanied by an increased intracellular accumulation of the fully processed virion structural proteins. In contrast, the levels of gp70 released into the medium were the same in the presence and absence of interferon. In control cells, gp70 can be either assembled into particles or released free into the cell culture fluid. However, gp70 present in the medium of interferon-treated cells was primarily nonpelletable, and little if any of the gp70 was found associated with virus particles.

The second major difference found was that no gp70/p12E complex was detected in either virions or tissue culture fluids from interferon-treated cells. The gp70/p12E complex can be readily demonstrated by reciprocal co-immunoprecipitation of untreated SC-1/MCF cells, since disulfide bonds between gp70 and p12E are maintained throughout the immunoprecipitation procedure, and the individual proteins are separated by reduction of the complex with β -mercaptoethanol before electrophoresis. There are two major interpretations of these data: first,

FIG. 8. Anti-gp70 immunoprecipitates of cell surface-iodinated cell extracts and particles released from control and interferon-treated cells. (1) Cell extract prepared immediately after iodination; (2) cell extracts after a 30-min chase; (3) particles after a 30-min chase; (4) cell extract after a 3-h chase; (5) particles after a 3-h chase.

that gp70 is particle associated in interferon-treated cells, but because of the lack of association with p15E/p12E is very susceptible to shear forces generated during centrifugation; and second, that gp70 is not assembled into particles and is released from p15E/p12E after proteolytic cleavage of gPr85^{env}. Several lines of evidence favor the latter hypothesis: (i) virus particles associated with the cells have a decreased infectivity (36); (ii) the amount of gp70 detected in virions is independent of virion preparation and purification; (iii) failure to detect a gp70/p12E complex in the medium before centrifugation; and (iv) the startling difference in the kinetics of release for gp70 compared with p30 and p15E/p12E.

The molecular basis for the failure of gp70 to be incorporated into retroviruses produced by interferon-treated cells is yet unknown. Maheshwari et al. (26) observed that interferon treatment of L-cells infected with vesicular stomatitis virus resulted in the production of vesicular stomatitis virus particles which had markedly reduced levels of the glycoprotein (G) and membrane protein (M). They also reported that [³H]glucosamine incorporation into the gp70 of Moloney leukemia virus particles was reduced (12). Maheshwari et al. later related these findings to an inhibition of the formation of asparagine-linked oligosaccharide chains, an effect similar to that induced upon exposure of cells to tunicamycin (6). We would like to suggest that since they analyzed only virus particles, the apparent lack of [³H]glucosamine-labeled envelope glycoprotein may have been due to a failure to incorporate viral glycoproteins into virions. In the SC-1/MCF system, as well as in other cells we have studied, no inhibition of N-linked glycosylation in the presence of interferon has been observed (J. Bilello and P. M. Pitha, manuscript in preparation). The envelope glycoprotein both from control and interferon-treated cells contains mannose, fucose, and glucosamine. Aboud et al. (1) recently reported an early decrease in the rate of [³H]glucosamine incorporation into the envelope glycoprotein precursor in interferon-treated cells. Although we have not been able to confirm these observations, there may be differences in methodology and the cell system studied. One possible mechanism for the failure to incorporate gp70 into virus particles comes from nucleotide and amino acid sequence analysis of gp70 (39; S. Oroszlan, personal communication), where it appears that the majority of the carbohydrate acceptor sites are at the carboxy-terminal end of gp70. It is conceivable that an altered carbohydrate side chain could interfere with the formation or the stability of disulfide bonds between gp70 and p15E/p12E, allowing the release of gp70 upon

proteolytic cleavage of gPr85^{env}. Alternatively, conformation changes at the membrane either due to interferon-induced alterations in gp70 or membrane stiffening (11, 30) may prevent the stable association between gp70 and p15E/p12E or lead to an increased release of gp70 at points distal to viral maturation sites. Experiments are in progress to distinguish between these alternatives.

Apart from their effect on virus replication, interferons affect a number of cellular functions and processes. Although we have found no interferon-induced alteration in the expression of gp70 at the cell surface, a number of studies have indicated that interferon alters the expression of histocompatibility and other antigens (for review, see reference 41). We also have found no alteration in the total amount of gp70 released or in the kinetics of this process, whereas others have reported changes in the release of plasminogen activator (20, 38) and carcinoembryonic antigen (2). We would like to stress that our observations on cell surface expression and release of gp70 apply only to this system. The studies of plasminogen activator release alluded to above have indicated that interferon can either potentiate or inhibit the release of a single protein, depending on the cell type or system studied.

Studies of the membrane budding virus vesicular stomatitis virus have indicated that specific host cell surface proteins can be incorporated into virions even in the absence of the viral envelope (G) glycoprotein (25). We have noted that after treatment of SC-1/MCF-infected cells with interferon, there is a temporal difference in the accumulation of a cellular 85-kilodalton glycoprotein into virions. This further strengthens our suggestion that the restriction in virus assembly is not selectively localized at the virus structural proteins, but may be a consequence of an interferon-induced change in plasma membrane of the infected cells, e.g., a decrease in fluidity.

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