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The Errors in Assembly of MuLV in Interferon Treated Cells

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Interferon treatment of JLSV-6 cells chronically infected with Rauscher MuLV leads to the formation of noninfectious particles (interferon virions) containing the structural proteins of env and gag genes as well as additional viral polypeptides. In the control virions the major glycoprotein detected is gp71, interferon virions contain in addition to gp71 and 85k dalton (gp85) glucosamine-containing, fucose-deficient glycoprotein which is recognized by antiserum to MuLV but not by the gp71 antiserum. The surface iodination of the intact virions indicates that both gp71 and gp85 are the major components of the external virions envelope. However, unlike the control virions in which gp71 associates with p15E (gp90), the gp71-p15E complex was not detected in interferon virions. The analysis of the iodinated proteins of the disrupted interferon virions revealed the presence of 85k and 65k dalton polypeptides preciptable with antiserum against MuLV, which are not present in the control virions. The difference in the polypeptide pattern of virions produced in the presence of interferon does not seem to be a consequence of the slowdown in the synthesis of viral proteins or their processing in the interferon-treated cells. Both the structural proteins of env and gag genes seem to be synthesized and processed at a comparable rate in the interferon-treated and -untreated cells. These results indicate an alteration of virus assembly in the presence of interferon.

Key words: MuLV, uninfectious particles, interferon, virus assembly

The several mechanisms by which interferon inhibits virus replication in an infected cell is through the impairment of mRNA translation [1–3]. In murine leukemia virus (MuLV) inhibition of viral replication occurs after the synthesis of viral RNA [4–7] and viral proteins [7–10] and seems to be mediated through changes in the cellular membrane [11, 12] and consequent interference with virus assembly and maturation [13–16]. Er-

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roneous assembly is reflected in some systems by the formation of noninfectious MuLV particles [5, 7, 17], and the virus particles accumulated on the cell surface of interferon-treated cells were shown to be less thermostable than the control virus [16]. In the AKR virus system, no noticeable morphologic difference was observed between the appearance of the particles from interferon-treated cells and controls [16], while interferon treatment of Friend erythroleukemia cells led to the formation of aberrant virus particles [18]. Virus particles produced in the presence or absence of interferon contain comparable amounts of 70S RNA [16], which indicates that the decrease in infectivity is not caused by the absence of the viral genome.

We now report that in the presence of interferon, MuLV is produced with abnormal structural proteins. By a combination of a high-resolution sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) and immunoprecipitation with antisera against viral proteins of the gag and env genes, we identified distinct groups of viral polypeptides of both high and low molecular weight in virions produced in the presence of interferon, but not in virions produced in the absence of interferon. Under these conditions, however, the synthesis of viral proteins in the interferon-treated cells was not altered.

METHODS

Cells and Viruses

Rauscher leukemia virus was obtained from chronically infected JLSV-6 cells (derived from BALB/c mouse bone) at titers of 2×10^6 plaque-forming units (PFU) per milliliter, as assayed by the UV-XC test in SC-1 cells [19]. Cells were grown in minimal essential medium (MEM) with Earle's salts containing 10% fetal bovine serum (FBS) and antibiotics. Treatment of the cells with 150 units/ml of interferon reduced the amount of infectious virus 20-fold to 50-fold, while the amount of virus particles in the medium (quantitated by reverse transcriptase activity or by uridine labeling) decreased five-fold.

Radioisotopic Labeling of Virus Particles and Cells

Several different labeling procedures were utilized depending on subsequent analysis of the proteins. Confluent cells chronically infected with MuLV were labeled with ^{3}H leucine (15 h) in leucine-free medium containing 10 μCi/ml of ^{3}H leucine in the presence of 5% dialized serum. To label intracellular viral proteins cells were labeled with interferon for 24 h prior to labeling and then incubated in amino acid-free medium for 30 min, then labeled for 20 min with ^{3}H amino acid mixture (100 μCi/ml) in MEM. Radioactivity was removed, and cells were washed and incubated in MEM with 5% FBS and interferon for an additional 3 or 6 h. Cells were harvested by scraping, and radioactive proteins were analyzed before and after immunoprecipitation on SDS gels.

Virus Purification

The labeled virus was purified from the medium by a two-step gradient technique described previously [16]. In principle the harvested medium was clarified at 10,000g for 10 min and the supernatant was filtrated through a 0.4-μ Millipore filter to remove cell debris and banded at the interface of 20% (w/v) sucrose and 40% (w/v) potassium tartrate in standard buffer. The banded virus was then centrifuged to equilibrium on a continuous sucrose gradient (24–48%, w/v). Fractions containing the virus were diluted with standard buffer and virus was pelleted at 340,000g for 1 h.

The unlabeled virus used for subsequent iodination was purified by the Sepharose C14B chromatographic method of McGrath et al [21]. In this method gp71 is preserved in the virus particle to a higher degree than by centrifugation. This seems to be especially important in the purification of virus assembled in the presence of interferon, which is fragile and easily loses gp71 during the centrifugation (Pitha, unpublished). Briefly, 12-h collections of culture fluids were clarified, and cell debris was removed by filtration on 0.4-μ filters and concentrated with immersible molecular separators (Millipore Corp.), mixed with tracer amounts of ^{3}H uridine-labeled virus, and chromatographed on a column of Sepharose C14B (Pharmacia) at 4°C in TEN buffer (20 m M Tris, 1 m M EDTA, 0.1 M NaCl pH 7.5). Virus appeared in this void column; fractions containing the virus peak were monitored by optical density at both 260 and 280 nm. The OD_{260}/OD_{280} ratio of the purified virus ranged from 1.22 to 1.27.

Antisera

The antisera were kindly provided by R. Wilsnack, Huntington Laboratories, Brooklandville, Maryland. The Rauscher MuLV antiserum was obtained from a goat immunized with purified virus particles disrupted by treatment with Tweenether. Goat antisera to gp71 and p30 were prepared by immunization of the animals with purified gp71 and p30 from Rauscher MuLV. The titers measured by radioimmunoassay (50% binding) were: gp71 antiserum 10.2×10^4 when gp71 was used as an antigen and lower than 50 for p15E and p30; p30 antiserum 9.5×10^4 for p30 and lower than 50 for p10, p12, and gp71; RLV antiserum 9.6×10^4 measured for gp71 and 2×10^2 for p12. Antiserum to FBS was a gift from Dr. Stephen Kennel, Oak Ridge National Laboratories.

Immunoprecipitation

Labeled virions or cells were disrupted and immunoprecipitated in the presence of Nonidet P40 (1%) and sodium deoxycholate (0.5%) in 25 mM Tris-HCl buffer, Ph 8.0, and 50 mM NaCl. Volumes were adjusted to 200 μl with the same buffer, to which 1 μl of the indicated serum was added. Serum used was preabsorbed on a monolayer of the uninfected cells fixed with methanol. The mixture was incubated for 30 min at 37°C, then for 2 h at 4°C. The immune complexes were precipitated by adding 50 μl of a 10% suspension of Staphylococcus aureus, Cowan strain (American Type Culture Collection) and incubated for 30 min at room temperature.

The precipitates were pelleted in a Brinkmann microfuge and washed twice with 50 mM Tris, pH 7.5, 0.1 M NaCl, 0.5% NP-40, 2.4 M KCl, and once with 50 mM Tris, pH 7.5, 0.10 M NaCl, 0.1% Trition X-100, and 5 mM EDTA. After the final wash, 45 μl of a mixture of 0.0625 M Tris, pH 6.8%, SDS, and 10% glycerol was added to the pellet. Samples assayed under reducing conditions then received 5 μl of 0.5 M iodoacetamide (0.05 M final concentration). All samples were placed in a boiling water bath for 10 min prior to electrophoresis.

Separation and Identification of the Proteins

The labeled viral proteins were separated by polyacrylamide (10% or 13%) gel electrophoresis in the presence of 0.1% SDS [23] and stained with Coomassie Blue R 250. Gels containing ^{3}H-labeled proteins were impregnated with scintillator and dried, and radioactive bands were detected by scintillation autoradiography using Kodak RP Xomat film. Dried gels containing ^{125}I-labeled proteins were exposed to Kodak RP Xomat film in the presence of a fast tungstate intensifying screen.

Interferon

Interferon was a generous gift from Dr. E. Knight. It was produced in L cells by induction with MM virus and purified as described previously [22]. The specific activity of the interferon was 5×10^6 units/mg protein. The antiviral activity of interferon was standardized against the reference mouse interferon (NIH) that had an assigned activity of 12×10^3 units/ml. The amount of interferon used throughout this study was 150 units/ml.

RESULTS

Effect of Interferon on Structural Proteins of MuLV

The polypeptide patterns of virions produced in the presence of interferon and purified on discontinuous and continuous sucrose gradients were examined by SDS polyacrylamide gel electrophoresis, followed by autoradiography, and compared to those of control virus. Labeling with ^{3}H amino acids for 15 h in the presence and absence of interferon gave patterns characteristic of MuLV [23] (Fig. 1). The virions assembled in the

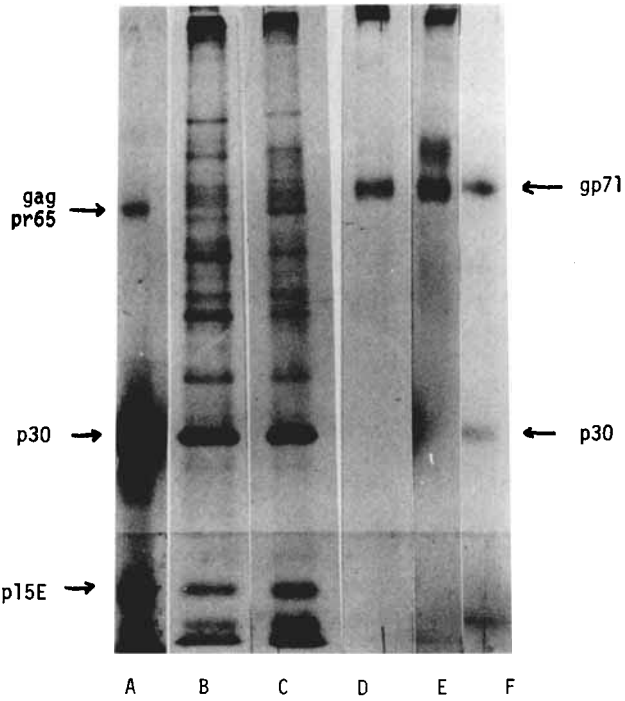

Fig. 1. Polypeptide pattern of MuLV synthesized in the presence and absence of interferon. JLSV-6 cells chronically infected with MuLV were grown without or with interferon (150 units/ml from 0 h to 40 h) and labeled (22–40 h) with 10 μCi/ml of ^{3}H amino acid mix. To label the virion glycoproteins with ^{3}H glucosamine, cells were incubated in glucose-free medium with 100 μCi/ml of ^{3}H glucosamine for 8 h (24–32 h) with or without interferon. Virus was purified by sucrose gradient centrifugation, disrupted, and analyzed on SDS PAGE as described in Methods. Approximately the same number of cpm of each sample were applied to the gel. ^{125}I-labeled gag pr65, p30, and p15 (A), ^{3}H amino acid-labeled proteins of control (B) and interferon (C) virions, ^{3}H glucosamine-labeled glycoproteins of control (D) and interferon (E) virions, and ^{125}I-labeled gp71 and p30 (F) are presented.

presence of interferon contained higher amounts of ^{3}H-labeled peptides which correspond in mobility to p15E and gag pr65 than the controls, while the amount of labeled p30 in the two types of virions was comparable. The interferon virions also have additional ^{3}H-labeled polydisperse bands present in the 72–90K, 40–50K, and 30–35K regions.

To determine whether the proteins detected in the 78–90K regions of the virions assembled in the presence of interferon represent the viral glycoproteins, MuLV was labeled with ^{3}H glucosamine for 24 h, and the glycopeptide patterns of the virions released both in the presence and absence of interferon were compared. SDS gels (Fig. 1) show a difference between the glycopeptide pattern of virions assembled in the presence and absence of interferon. In the control virions the main ^{3}H glucosamine-labeled band was gp71 (gp45 and gp32 were detected as minor bands only after much longer exposure), while the interferon virions contained an additional ^{3}H glucosamine containing 85K protein. This glycoprotein was not detected in virions labeled with fucose (data not shown). The gp45 and gp32 were detected only as minor bands seen after the longer exposure.

The new high-molecular-weight glycoprotein present in the virions from interferon-treated cells was precipitated with antiserum to disrupted MuLV but not with the anti-gp71 serum. Furthermore the precipitation with anti-MuLV serum revealed the presence of the high-molecular ^{3}H glucosamine-labeled glycoproteins not detected in unprecipitated, disrupted virions. These glycoproteins seem to be highly reactive with the MuLV serum. The presence of the high-molecular-weight glycoproteins was not affected by the degree of purification, and thus it is unlikely that these polypeptides represent cellular contamination.

While this work was in progress it was reported that MuLV produced in interferon-treated cells contains a 90K protein containing glucosamine; whether this is a viral or cellular protein has not been established [24].

Components of MuLV Reactive With Antiserum to MuLV, p30, and gp71

To determine the antigenic specificity of the proteins present in the interferon virions, MuLV produced in the presence of interferon was purified and disrupted with Triton X-100, and viral proteins were iodinated, analyzed, and compared to proteins present in the control virions.

The viral proteins were first precipitated with the gp71 antiserum to remove the envelope glycoprotein, and the rest of the ^{125}I-labeled protein was precipitated with MuLV antiserum, the precipitates being analyzed by SDS PAGE electrophoresis and autoradiography (Fig. 2). The antiserum to gp71 removed iodinated gp71 from the protein mixture of both control and interferon virions. In the control virus, the sequential precipitation with MuLV antiserum precipitated only the ^{125}I-labeled structural proteins of the gag gene (p30, p15, p10) and p15E. In interferon virions the MuLV antiserum precipitated, in addition, 85K and 65K proteins as major ^{125}I-labeled bands. These data indicate that the interferon virions contain at least two high-molecular-weight proteins (85K and 65K) which are not detected in the control virions. The 85K protein may be identical to gp85 detected in ^{3}H glucosamine-labeled interferon virions.

Differential Localization of Viral Glycoproteins in the Virion Membranes

It was shown previously [24] that lactoperoxidase treatment of intact MuLV labels predominantly the 78K viral protein, which is antigenically related to gp71. To examine how the presence of gp85 in the interferon virions affects their membrane topology, virions produced in the presence and absence of interferon were purified on a Sephadex C14B

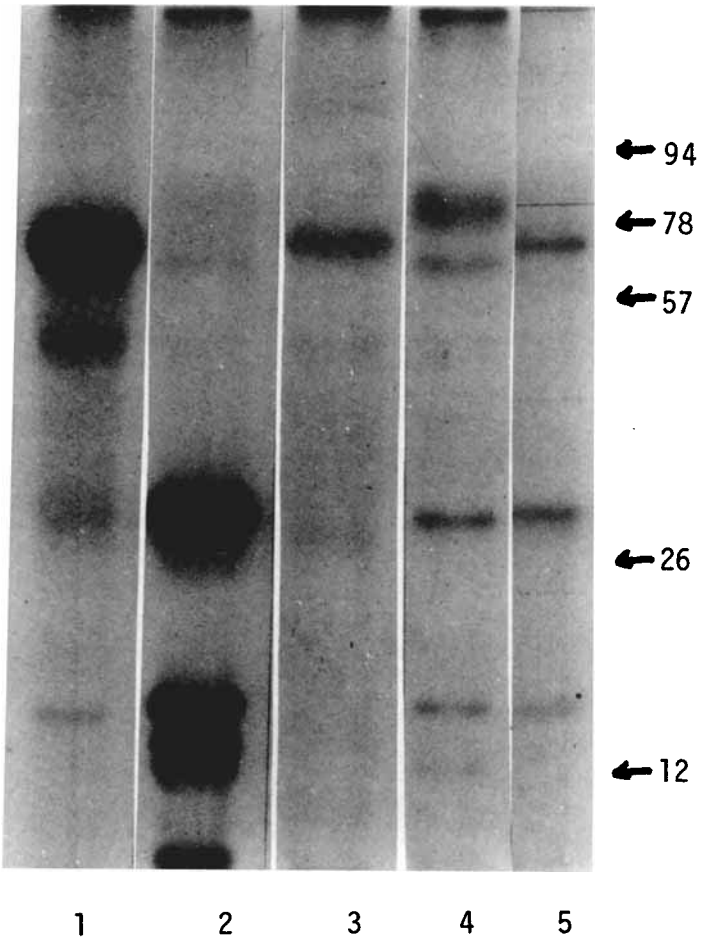

Fig. 2. Sequential immunoprecipitation and SDS PAGE gel electrophoresis of iodinated MuLV proteins detected in virions produced in the presence and absence of interferon. Virus produced in the presence and absence of interferon was harvested, purified, iodinated, and analyzed as described in Methods. Iodinated proteins (2×10^6 cpm) were precipitated first with anti-gp71 serum [control (1) and interferon (3)] and the supernatant was precipitated with anti-MuLV serum [control (2) and interferon (4)]. ^{125}I-labeled gp71, p30 and p15 (5).

column, iodinated by the solid-state lactoperoxidase method [20], disrupted, and treated with respective antiserum, and the precipitates were analyzed by polyacrylamide gel electrophoresis followed by autoradiography.

Surface iodination of the control virions revealed the presence of one major surface protein with the mobility of gp71 (Fig. 3), when assayed in the presence of mercaptoethanol. To confirm its relationship, immunoprecipitation was done with the gp71 antiserum. When the precipitate was analyzed under reducing conditions gp71 was detected as a major labeled band with minor bands in the gp45 and gp32 regions. The antiserum to p15(E) precipitated 90K but not gp71 (data not shown). The fact that no radioactive p15(E) was detected indicates that in the intact virions p15(E) is not accessible for surface iodination. No iodinated viral proteins were precipitated with p30 antiserum.

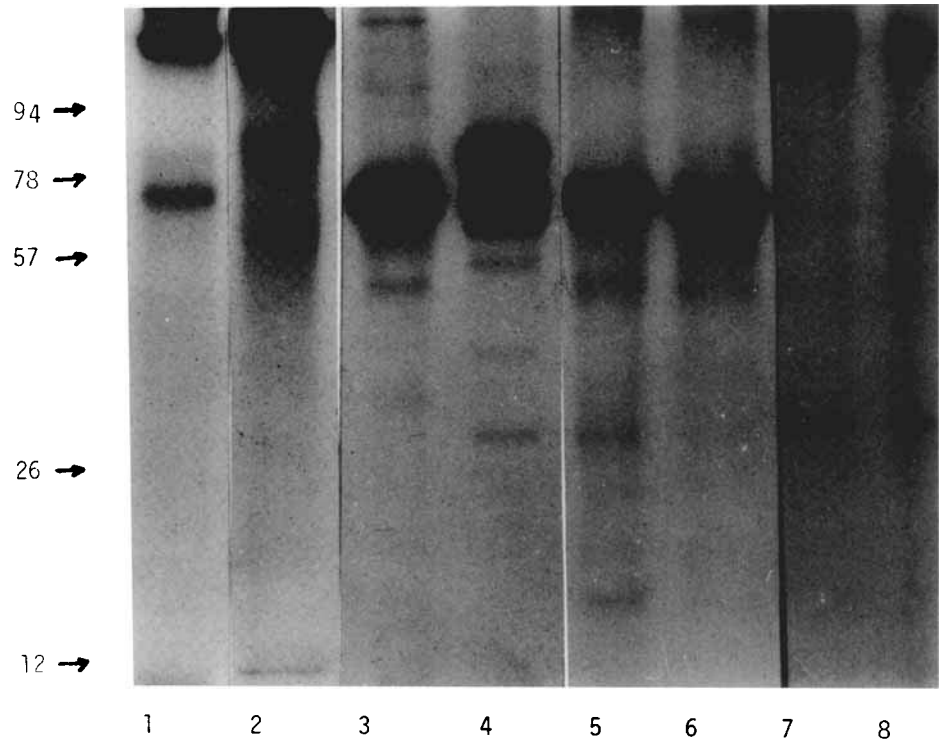

Fig. 3. Accessibility of viral glycoproteins on the surface of virions produced in the presence of interferon. Virions produced in interferon-treated cells (150 units/ml, interferon present 0–36 h, virions harvested 24–36 h) were purified, labeled, and analyzed as described in Methods. Unprecipitated samples (0.1 μg of protein) control virions (1) and interferon (2) virions. Immunoprecipitation of iodinated proteins (2×10^6 cpm); gp71 antiserum:controls reducing (3) nonreducing (4) conditions; interferon reducing (5) nonreducing (6) conditions; p30 antiserum:controls (7) interferon (8).

Surface iodination of the intact interferon virions revealed patterns different from those of the control virions (Fig. 3). Analysis of the iodinated proteins after precipitation with gp71 antiserum under both nonreducing and reducing conditions revealed only the presence of a gp71. Antiserum to p30 did not precipitate any labeled viral proteins. No precipitation of ^{125}I-labeled proteins was detected with the normal goat serum (data not shown). These data provide evidence that the mature MuLV produced in the presence and absence of interferon have different surface morphology.

Effect of Interferon on the Rate of Synthesis of Viral Proteins

We have shown previously that the synthesis of p30 and gp71 is not inhibited by the interferon treatment in AKR-2B cells chronically infected with MuLV [10]. These experiments, however, did not eliminate the possibility that in interferon-treated cells the rate of viral protein synthesis or the cleavage of the precursors of viral proteins is slowed down. To determine whether the observed lack of fidelity in the virus assembly could be explained by the slowdown in the rate of viral protein synthesis or its processing, the interferon-treated cells were pulsed for short periods of time with ^{3}H amino acids, and then the radioactivity was chased for 3 and 6 h in the presence of interferon. The ^{3}H-labeled proteins

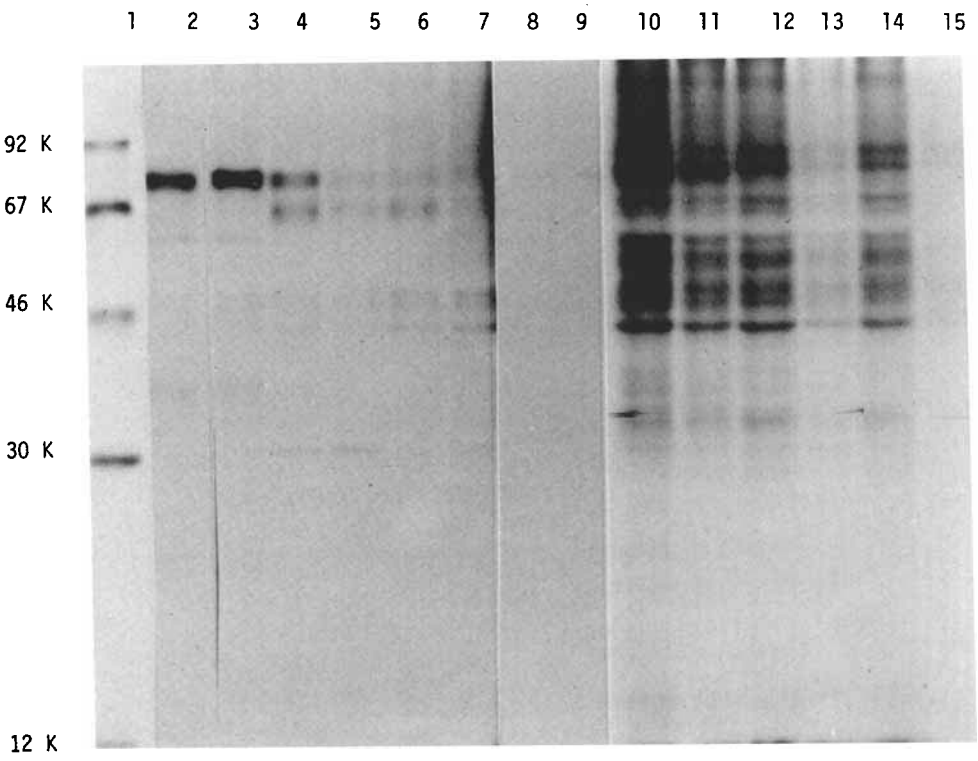

Fig. 4. Synthesis of viral proteins in the cells in the presence and absence of interferon. The cells were treated with interferon (for 24 hrs) and pulsed with ^{3}H amino acid (100 μCi/ml) for 20 min; the radioactivity was chased for 3 and 6 h. The ^{3}H-labeled proteins were analyzed before precipitation (10–15) (0.1 μg protein per well) and after precipitation (2–7) with anti-MuLV serum (10^{5} cpm/well). Controls: pulse (2, 10), 3-h chase (4, 12), and 6-h chase (6, 14). Interferon-treated cells: pulse (3, 11), 3-h chase (5, 13), and 6-h chase (7, 15). ^{125}I-labeled gp71 and p30 (1). Non immune serum pulse controls (8), interferon (9).

were analyzed on SDS PAGE before and after immunoprecipitation with anti-MuLV, gp71, and p30 serum. The analysis of the labeled proteins (Fig. 4, lanes 10–15) on acrylamide gels indicated that in interferon-treated cells the amount of proteins labeled in a 20-min pulse was lower than in the controls. This difference became much more obvious when the proteins were analyzed after a 3- and a 6-h chase, indicating faster turnover of labeled proteins in MuLV-infected, interferon-treated cells. No qualitative difference, however, was observed after immunoprecipitation (Fig. 4, lanes 2–7). In the cells labeled for a short period of time with ^{3}H amino acid in the presence and absence of interferon, the antiserum against MuLV precipitated env pr 85 and gag pr 65, and these were further processed into gp71 and p30, respectively, both in the interferon-treated cells and controls. We have shown that the virions from interferon-treated cells contain gp85, which is antigenically unrelated to gp71 but is recognized by the anti-MuLV serum. In order to determine whether the env pr 85 and gag pr 65 synthesized in the cells show antigenic similarity to gp71 and p30, the ^{3}H-labeled proteins from interferon-treated cells and controls were precipitated with anti-gp71 and anti-p30 serum and analyzed on SDS PAGE. The results, in Figure 5, indicate that the env pr 85 present in the interferon-treated cells is antigenically related to gp71 and there-

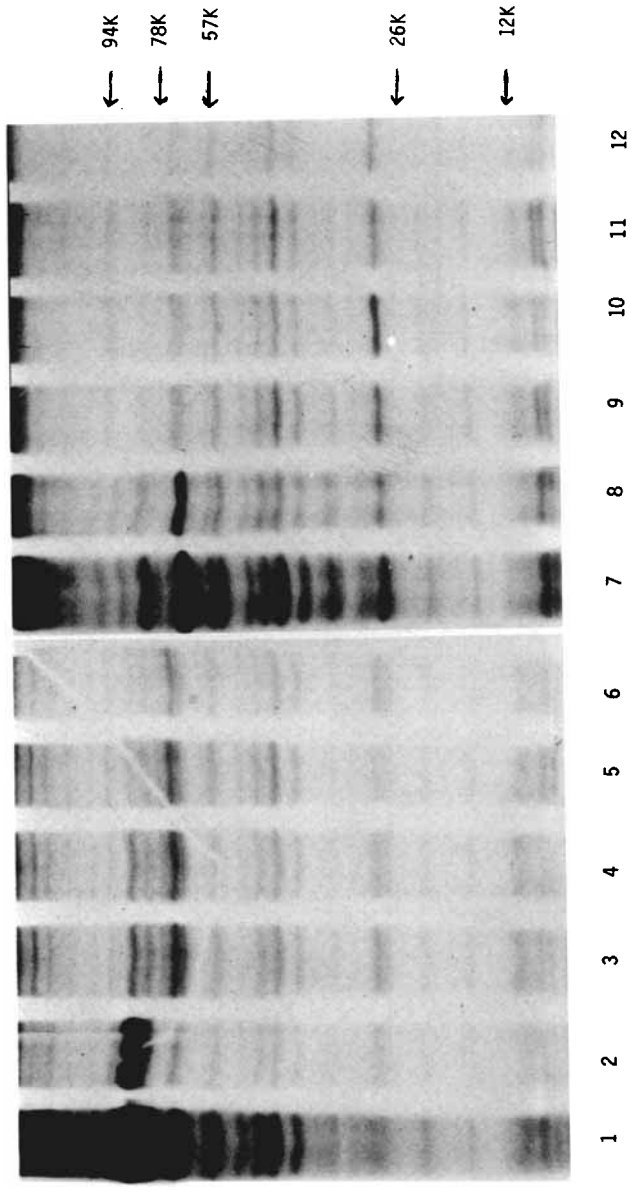

Fig. 5. Synthesis and processing of viral proteins in interferon-treated cells and controls. Cells were labeled as described in Figure 4 and viral proteins were precipitated with anti-gp71 serum (1–6) and anti-p30 serum (7–12). Controls: pulse (1, 7), 3-h chase (3, 9), and 6-h chase (5, 11). Interferon-treated cells: pulse (2, 8), 3-h chase (4, 10), and 6-h chase (6, 12).

fore probably represents a different protein from that present in the virions. Similarly, the gag pr 65 present in the interferon-treated cells has antigenic similarity to p30.

These results indicate that the rates of synthesis and processing of viral proteins in interferon-treated cells are comparable to those in the control cells.

Finally, we examined the possibility that the interferon treatment leads to a redistribution of the viral proteins in the plasma membrane and thus to erroneous assembly. The surface membrane proteins of the interferon-treated cells and controls were selectively labeled with ^{125}I iodine, the cells were lysed, and viral proteins were analyzed after precipitation with anti-gp71 serum. The results, in Figure 6, indicate that both in the interferon-treated and in the untreated cells the major protein labeled was gp71, and that this protein survived under nonreducing conditions as gp90. Thus, in the interferon-treated cells as well as in the control the gp71 glycoprotein is a major viral surface protein.

DISCUSSION

The results presented indicate that interferon treatment of JLSV-6 cells persistently infected with MuLV leads to the formation of virus particles containing several novel polypeptides not detected in the control virions.

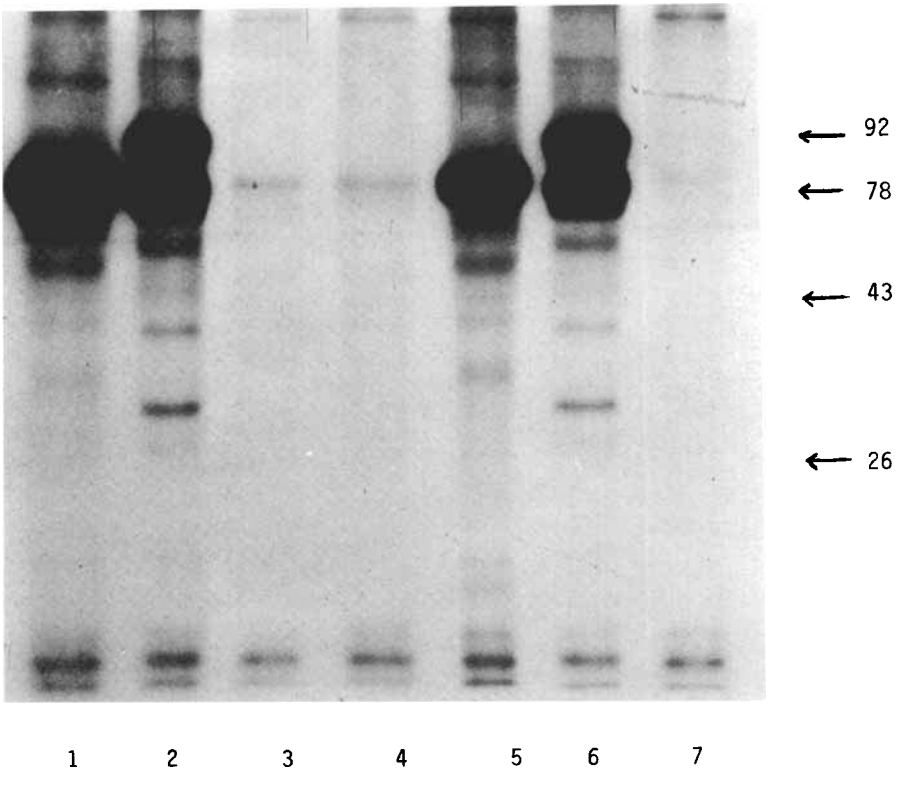

Fig. 6. The surface ^{125}I-labeling of infected cells grown in the presence and absence of interferon. The surface of the cell was labeled as described in Methods. Viral proteins were precipitated with anti-gp71 serum (1, 2, 5, 6) and anti-p30 serum (3, 7) and analyzed on SDS PAGE in reducing (1, 3, 5, 7) and nonreducing (2, 6) conditions. Controls:anti-gp71 serum (1, 2); anti-p30 serum (3); normal goat serum (4); interferon-treated cells; anti-gp71 serum (5, 6); anti-p30 serum (7).

Surface iodination of intact virions revealed that in interferon-treated virions both 85k(gp85) and gp71 are major components of the virions envelope. Unlike the control virus, in which gp71 (a major membrane protein) can associate through a disulfide bond linkage to p15E [25], the gp71-p15E complex was not detected in the virions assembled in the presence of interferon.

The analysis of the virion proteins iodinated after virus disruption indicated that the interferon-treated virion contain, in addition to gp85, a 65k protein which was not seen in the control virus. It is particularly interesting that these two peptides in interferon-treated virions are precipitable with antiserum against MuLV; however, these proteins are not efficiently recognized by the gp71 and p30 antiserum. The question whether the new proteins in the interferon virions are coded by the env and gag genes needs further investigation. The post transcriptional modifications of the gene products may be altered in interferon-treated cells and this may change the antigenicity of the products. Further identification of these proteins by fingerprinting or partial digest analysis should be able to determine their relationship to the env pr85 and gag pr65. The possibility that the new proteins are the cellular proteins or viral nonvirion proteins also cannot be completely eliminated. It has been shown recently that vesicular stomatitis virus assembles several cellular glycoproteins into the virions (Lodish, personal communication). If a similar situation happens with MuLV, then the antiserum to disrupted MuLV would contain the antibodies to cellular glycoproteins as well. Since the activity of the antiserum to MuLV to precipitate these proteins was not removed by preadsorption with fixed uninfected cells, this would indicate that these proteins detected in the interferon virions are not present on the membranes of the uninfected cells.

Retrovirus assembly and maturation are membrane-associated events. It is assumed that a cleavage of the precursors of the gag proteins occurs at the time of virus budding or core formation [26]. Previous work [27, 28] indicates that the precursors of the gag proteins are detectable in extracellular virus particles; this may indicate that only a partial cleavage of gag pr65 is required for virus release and that a final maturation of the core is coupled with further cleavage. Proteolytic activity associated with Rauscher MuLV has been shown to cleave gag pr 65 into the lower-molecular-weight structural proteins [26]. Accordingly, the amount of p30 detected in the virions released from interferon-treated cells seems to be time-dependent (data not shown).

An alternative explanation would be that interferon affects post transcriptional modification and processing of one or several primary gene products. Failure of the processing of env and gag precursors in interferon-treated cells could lead to insertion of these proteins into virions together with the fully processed viral proteins. However, the intracellular synthesis and processing of precursor by viral proteins does not seem to be affected by interferon treatment.

Thus the molecular basis of the erroneous virus assembly in interferon-treated cells remains unknown. However, the finding that interferon virions contain significantly more high-molecular-weight proteins than the controls and the fact that in interferon-treated cells mature noninfectious virus particles accumulate on the cell surface [9, 14–16, 30]. indicate that the erroneous assembly leads both to the inhibition of virus release and a decrease in its infectivity.

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