Effect of Interferon on Mouse Leukaemia Virus (MuLV).

V. Abnormal Proteins in Virions of Rauscher MuLV
Produced in the Presence of Interferon

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SUMMARY

Interferon treatment of JLSV-6 cells chronically infected with Rauscher MuLV
leads to the formation of non-infectious particles (‘interferon’ virions) containing
the structural proteins coded by the env and gag genes as well as additional virus
polypeptides. The major glycoprotein detected in the control virions is gp71, but
‘interferon’ virions contain in addition an 85K mol. wt. (gp85) glucosamine-
containing, fucose-deficient glycoprotein. This is recognized by antiserum to
MuLV and may be related to env pr85. Surface iodination of intact virions in-
dicates that gp71 and gp85 are the two major components of the external envelope.
However, whereas in control virions gp71 associates with p15E (gp90), this complex
was not detected in ‘interferon’ virions. Analysis of radio-labelled (3H-amino acids
or iodinated) proteins from disrupted ‘interferon’ virions revealed the presence of
65K, 55K, 40K, 20K and 12K mol. wt. polypeptides which could be precipitated
with antiserum against MuLV. There was a distinct difference in the patterns of in-
corporation of pulse-labelled 3H-amino acid polypeptides into virions in the
presence and absence of interferon. Those polypeptides labelled in the presence
of interferon and recovered in the extracellular virions in a chase with interferon
appeared to have substantially fewer copies of p30 and more of gag pr55 poly-
peptide than the controls. These results indicate that in the presence of interferon
there are changes in the proteolytic cleavage associated with virion assembly.

INTRODUCTION

Although the mechanism by which interferon inhibits virus replication in an infected cell
has not been fully clarified, there is evidence with most viruses of impaired mRNA trans-
lation (Joklik & Merigan, 1966; Jungwirth et al. 1972; Metz & Esteban, 1972). However,
with murine leukaemia virus (MuLV), inhibition of virus replication is inhibited after
there has been synthesis of virus RNA (Billiau et al. 1977; Pitha et al. 1977; Aboud et al.
1978; Fan & MacIsaac, 1978) and most virus proteins (Friedman & Ramseur, 1974; Fried-
man et al. 1975; Pitha et al. 1976, 1977); it seems to be mediated through changes in the
cellular membrane (Friedman, 1977; Pitha & Rowe, 1977) with consequent interference
with virus assembly and maturation (Billiau et al. 1974; Chang et al. 1977; Billiau et al.
1978; Pitha et al. 1978). Erroneous assembly is reflected in some systems by the formation
of non-infectious MuLV particles (Pitha et al. 1976; Wong et al. 1977; Billiau et al. 1978)
and the virus particles accumulated on the surface of interferon-treated cells are less thermostable than the control virus (Pitha et al. 1978). In the AKR virus system, there was no noticeable difference between the appearance of particles from interferon-treated cells and controls (Pitha et al. 1978), while interferon treatment of Friend erythroleukaemia cells led to the formation of aberrant virus particles (Luftig et al. 1977). Virus particles produced in the presence or absence of interferon contain comparable amounts of 70S RNA (Pitha et al. 1978), so that the decrease in infectivity is not caused by the absence of the virus genome. However, although it is not likely that a defect in one or more glycoprotein moieties would be detected on the ultrastructural level, such a defect could account for lack of virus infectivity and lower thermal stability. Accordingly, we have examined and compared the peptide composition of virions released in the presence ('interferon' virions) and absence (control virions) of interferon. 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 sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoprecipitation with antisera against the virus proteins encoded by the \textit{gag} and \textit{env} genes, we have identified characteristic groups of virus polypeptides of both high and low mol. wt. in 'interferon' virions which are not present in control virions. The implications of these results are discussed.

\section*{Methods}

\textit{Cells and viruses.} Rauscher leukaemia virus was obtained from chronically infected JLSV-6 cells (derived from Balb/c mouse bone marrow) at titres of $2 \times 10^6$ p.f.u./ml as assayed by the UV-XC test in SC-1 cells (Hartley & Rowe, 1975). Cells were grown in Eagle's minimal essential medium (MEM) with Earle's salts containing 10\% foetal bovine serum (FBS) and antibiotics. Treatment of the cells with 150 units/ml of interferon reduced the amount of infectious virus 20- to 50-fold, while the amount of virus particles in the medium (measured by reverse transcriptase activity or by uridine labelling) decreased fivefold.

\textit{Radioisotopic labelling of virus particles.} Several different labelling procedures were utilized depending on subsequent analysis of the proteins. Confluent cells chronically infected with MuLV were incubated for 15 h in leucine-free medium containing 10 $\mu$Ci/ml of $^3$H-leucine (15 Ci/ml, New England Nuclear) in the presence of 5\% dialysed serum. In the pulse-chase of $^3$H-leucine into completed virions, cells were grown either in the presence or absence of interferon and pulsed for 30 min with $^3$H-leucine (50 $\mu$Ci/ml) in leucine-free medium without serum. They were then washed and chased with MEM with 5\% FBS with and without interferon, and medium was collected for 3 h periods at 0 to 3, 3 to 6 and 6 to 9 h. To label virion glycoproteins with $^3$H-glucosamine, cells were incubated in glucose-free medium with 100 $\mu$Ci/ml of $^3$H-glucosamine (New England Nuclear).

The solid state lactoperoxidase method (Kennel & Lerner, 1973) was used to iodinate proteins exposed on the external virus envelope and those exposed after virion disruption (treatment for 30 min at 37\°C in 0-1\% Triton X-100). The iodinated intact virions were separated at 4\°C from free $^{125}$I on a Sepharose C14B column (McGrath et al. 1978). The iodinated proteins of the disrupted virions were separated from free $^{125}$I at room temperature on Sephadex G25 (Pharmacia), equilibrated in TEN buffer (20 mM-tris, 1 mM-EDTA, 0.1 M-\textit{NaCl}, pH 7.5) and 0.1\% Triton X-100.

\textit{Virus purification.} Labelled virus was purified by the two-step gradient technique described previously (Pitha et al. 1978). Briefly, the harvested medium was clarified at 10000 \textit{g} for 10 min and the virus in the supernatant was banded at the interface of 20\% (w/v) sucrose and 40\% (w/v) potassium tartrate in standard buffer (Duesberg et al. 1968). The banded virus was then centrifuged to equilibrium on a continuous sucrose gradient (24 to 48\%, w/v).
Fractions containing the virus were diluted with standard buffer, and virus was pelleted at 34,000 g for 1 h.

The unlabelled virus used for subsequent iodination was purified by a Sepharose C14B chromatographic method (McGrath et al. 1978), in which more gp71 is preserved in the virus particle than during centrifugation. This seems to be especially important for virus assembled in the presence of interferon, since this is fragile and easily loses gp71 during centrifugation (P. M. Pitha, unpublished data). Briefly, culture fluids, collected during a 12 h period were clarified, concentrated with immersible molecular separators (Millipore Corp. Bedford, Mass.), mixed with tracer amounts of ³H-uridine labelled virus and chromatographed on a column of Sepharose C14B (Pharmacia) at 4 °C in TEN buffer. Virus appeared in the void column; absorbances of the fractions containing the virus peak were monitored at both 260 and 280 nm. The $A_{260}/A_{280}$ ratio of the purified virus ranged from 1.22 to 1.27.

**Antisera.** Rauscher MuLV antiserum was obtained from a goat immunized with purified virus particles disrupted by treatment with Tween-ether. Antiserum to gp71 and p30 were prepared by immunization of goats with purified gp71 and p30 from Rauscher MuLV. These antisera were kindly provided by R. Wilsnack, Huntington Laboratories, Brooklandville, Md. The 50% binding titres measured by radioimmunoassay were: gp71 antiserum, $10^{2} \times 10^{4}$ with gp71 as an antigen and < 50 with p15E and p30; p30 antiserum, $9.5 \times 10^{4}$ with p30 and < 50 for p10, p12 and gp71; RLV antiserum, $9.6 \times 10^{4}$ with gp71 and $2 \times 10^{2}$ with p12. Antiserum to FBS was a gift from Dr Stephen Kennel, Oak Ridge National Laboratories, Tenn.

**Immunoprecipitation.** Labelled virions 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. The mixture was incubated for 30 min at 37 °C and 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 incubating 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% NP40, 2.4 M-KCl and once with 50 mM-tris, pH 7.5, 0.10 M-NaCl, 0.1% Triton X-100 and 5 mM-EDTA. After the final wash, 45 μl of a mixture of 0.0625 mM-tris, pH 6.8, 2% SDS and 10% glycerol were added to the pellet. Samples assayed under reducing conditions were then treated with 2-mercaptoethanol (1.25% final concentration) and those assayed under non-reducing conditions with iodoacetamide (0.05 M final concentration). All samples were placed in a boiling water bath for 10 min before electrophoresis.

**Separation and identification of the proteins.** The labelled virus proteins were separated by polyacrylamide (10 or 13%) gel electrophoresis in the presence of 0.1% SDS (Laemmli, 1970) and stained with Coomassie Blue R 250. Gels containing ³H-labelled proteins were impregnated with scintillator and dried, and radioactive bands were detected by scintillation autoradiography using Kodak RP X-Omat film (Bonner & Laskey, 1974). Dried gels containing ¹²⁵I-labelled proteins were exposed to Kodak RP X-Omat film in the presence of a fast tungstate intensifying screen. Autoradiographs were scanned on a Clifford Densitometer, model 445. The mol. wt. estimates were interpreted from the mobility of the standard proteins, phosphorylase (94K) and pyruvate kinase (57K).

**Interferon.** Mouse interferon with asp. act. of $5 \times 10^{6}$ units/mg protein was a generous gift from Dr E. Knight. It was produced in L cells by induction with MM virus and purified as described previously (Knight, 1975). The amount of interferon used throughout this study was 150 research reference units/ml (in terms of the National Institutes of Health mouse interferon Research Reference Standard, Catalogue no. Go02-904-511).
Fig. 1. Polypeptide patterns 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 labelled from 22 to 40 h with 10 μCi/ml of 3H-amino acid mix, or with 100 μCi/ml of 3H-glucosamine from 24 to 32 h. Virus was purified by sucrose gradient centrifugation, disrupted and analysed by SDS-PAGE as described in Methods. Approximately the same number of ct/min of each sample was applied to each gel. (a) 125I-labelled gag pr65, p30 and p15E. (b) and (c) 3H-amino acid labelled proteins of control and interferon virions; (d) and (e) 3H-glucosamine labelled glycoproteins of control and interferon virions; (f) 125I-labelled gp71 and p30.

RESULTS

The effect of interferon on structural proteins of MuLV

The polypeptide patterns of virions produced in the presence or absence of interferon and purified on discontinuous and continuous sucrose gradients were examined by SDS polyacrylamide gel electrophoresis (PAGE), followed by autoradiography. Labelling with 3H-amino acids for 15 h in the presence and absence of interferon gave patterns characteristic of MuLV (Strand & August, 1976; Fig. 1 b, c). The virions assembled in the presence of interferon contained higher amounts of those 3H-labelled peptides which correspond in mobility to p15E and gag pr65 than the controls, but comparable amounts of labelled p30. The interferon virions also contained additional 3H-labelled poly-disperse bands in the 72 to 90K, 40 to 50K and 30 to 35K regions. Since virions produced in the presence of interferon are more thermolabile than control virions (Pitha et al. 1978) and more fragile on sucrose gradient purification, the polypeptide pattern of virions which had not been exposed to osmotic shock was also examined. Virions labelled for 18 h with 3H-amino acid were pelleted from the culture fluids (100000 g for 1 h), disrupted, precipitated with RLV antiserum and analysed on SDS-PAGE. The patterns obtained were similar to those of sucrose gradient purified virions (data not shown).

To determine whether the proteins detected in the 78 to 90K region of the interferon
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Fig. 2. Densitometric scans of MuLV glycoproteins produced in the absence or presence of interferon (150 units/ml from 0 to 32 h). Virions were labelled with 3H-glucosamine (100 µCi/ml) from 24 to 32 h, and at the end of the labelling period, the virions in the medium were purified by sucrose gradient centrifugation and their glycoproteins analysed on SDS-PAGE as described in Methods. (a) Control and (b) interferon virions. (c) The cells were incubated for an additional 16 h in medium without interferon (interferon-free chase) after which virus was similarly isolated and assayed. (d to f) Virus proteins were radioimmunoprecipitated with anti-MuLV serum as described in Methods. (d) Control and (e) 'interferon' virions; (f) virions from interferon-free chase. The absorbance arbitrary units are the same on all scans.

virions represent the virus glycoproteins, MuLV was labelled with 3H-glucosamine for 8 h and the glycopeptide patterns of the virions released both in the absence and presence of interferon were compared. Differences were found in SDS gels (Fig. 1 d, e) and densitometric scans (Fig. 2 a, b). In the control virions, the main 3H-glucosamine labelled band was gp71, while the 'interferon' virions contained an additional 3H-glucosamine-containing 85K protein. This glycoprotein was not detected in virions labelled with fucose (data not shown). In both control and 'interferon' virions gp45 and gp32 were detected only as minor
bands seen after the longer exposure. The continuous presence of interferon seems to be required for the persistence of gp85 in the MuLV, since the amount of gp85 was decreased in virions which were labelled in the presence of interferon, but harvested after a 16 h chase without interferon (Fig. 2c). At this time the number of virus particles (measured in terms of the virion-associated reverse transcriptase activity) recovered in the medium was only 35% of the control value and the infectivity (measured by the UV-XC test) was only 10%. However, production of infectious virus in this cell line returned to normal levels within 48 h after removal of interferon, which confirms our previous finding that the interferon-induced inhibition of virus release is only temporary (Pitha et al. 1976).

To determine whether the gp85 present in the virions from interferon-treated cells was viral or cellular protein, the virion glycoproteins, labelled with 3H-glucosamine, were precipitated with MuLV antiserum and analysed (Fig. 2d to f). In the control virions (Fig. 2a, d) gp71 was the predominant component of the precipitate. In the virions produced in the presence of interferon both gp85 and gp71 were precipitated (Fig. 2b, e); in addition, the precipitate contained both high mol. wt. and low mol. wt. 3H-glucosamine labelled proteins (Naso et al. 1976). In the immunoprecipitate of virus produced during the chase without interferon (Fig. 2c, f) both gp95 and the high mol. wt. glycoproteins seen in the interferon virions were less abundant.

These results indicate that the new high mol. wt. glycoprotein present in the virions from interferon-treated cells is a virus-related protein. The fact that the gp85 is fucose-deficient suggests that it may be related to env pr85, previously detected in cells infected with MuLV and shown to be the precursor of gp71 and p15E proteins (Naso et al. 1975, 1976). Furthermore, the precipitation with MuLV serum revealed the presence of high mol. wt. 3H-glucosamine labelled glycoproteins which were not detected in the unprecipitated, disrupted virions and seem to be highly reactive with the MuLV serum. 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 was not established (Chang & Friedman, 1977).

Components of MuLV reactive with antiserum to MuLV, p30 and gp71

To determine the antigenic specificity of the proteins present in the control and ‘interferon’ virions, MuLV produced in the presence and absence of interferon was purified and disrupted with Triton X-100 and the virus proteins were iodinated, precipitated with the specific antiserum and analysed. In the control virions (Fig. 3, lane 1) the antiserum to MuLV precipitated the major 125I-labelled virion proteins – gp71, p30, p15, p12, p15E, and p10. Apart from the structural proteins, a minor 125I-labelled band with a mobility of 55000 was detected. After precipitation with gp71 antiserum, only gp71 was selected as a major 125I-labelled band under reducing and non-reducing conditions (Fig. 3, lanes 2, 3); p30 antiserum precipitated p30 as a major 125I-labelled band and p15 as a minor band (Fig. 3, lanes 4, 5); the antiserum to normal goat serum did not precipitate any virus proteins.

When ‘interferon’ virions were similarly analysed, the pattern of 125I-labelled proteins precipitated with MuLV antiserum was more complex (Fig. 3, lane 9). In addition to the major structural proteins, many 125I-labelled bands were detected (120K, 90K, 85K, 65K, 55K, 40K, 35K, 28K, 18K, 12K), which were not seen in the control virions. The p30 antiserum precipitated 125I-labelled p30 as a major component (Fig. 3, lanes 12, 13). Antiserum to gp71 precipitated gp71 as a major band (Fig. 3, lanes 10, 11), but much less than was precipitated with MuLV antiserum. Also with the same amount of radioactivity (1 x 10⁶ ct/min), the counts precipitated from disrupted interferon virions with gp71 antiserum were half as many as from the controls; however, when the precipitate was analysed on SDS gels, the amount of 125I-labelled gp71 detected in interferon virions was only 15% of
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Fig. 3. SDS–PAGE electrophoresis of iodinated virus proteins. Virions produced in the absence (lanes 1 to 6) and presence (lanes 9 to 14) of interferon (150 units/ml for 0 to 36 h) were harvested between 24 and 36 h, purified on Sepharose C14B column and disrupted. The virus proteins (0.5 µg) were iodinated with 125I and 10⁶ cts/min were precipitated with antiserum. The precipitate was analysed by SDS–PAGE and fluorography under reducing (lanes 2, 4, 10, 12) and non-reducing (lanes 3, 5, 11, 13) conditions as described in Methods. The antisera used were directed against MuLV (lanes 1 and 9); gp71 (lanes 2, 3, 10, 11); and p30 (4, 5, 12, 13). Controls: normal goat serum (lane 6) and ‘interferon’ virions (lane 14). Mol. wt. standards included standard proteins (1 µg of each) phosphorylase (94K), human transferrin (78K), pyruvate kinase (57K), chymotrypsinogen (26K), cytochrome c (12K) and 125I-labelled p30, p15, p12 (lane 7) and gp71 (lane 8).

that in the controls. The efficiency of protein precipitation was not altered by the presence of interferon in the reaction mixture.

To identify the minor virus polypeptides in the virions, sequential immunoprecipitation of the iodinated proteins was performed (Fig. 4). The virus proteins iodinated after virus disruption were first precipitated with the gp71 antiserum to remove the envelope glycoproteins and then the rest of the 125I-labelled proteins was precipitated with the MuLV antiserum. Both precipitates were analysed by SDS–PAGE electrophoresis and autoradiography. The antiserum to gp71 removed iodinated gp71 from the mixed proteins of both control (Fig. 4, lane 1) and interferon virions (Fig. 4, lane 3). With the control virus the sequential precipitation with MuLV antiserum (Fig. 4, lane 2) precipitated only the labelled structural proteins of the gag gene (p30, p15, p10) and p15E. With ‘interferon’ virions the MuLV antiserum precipitated (Fig. 4, lane 4), in addition to the labelled structural proteins of the gag gene, 85K and 65K proteins as major iodinated bands. These data indicate that the ‘interferon’ virions contain at least two high mol. wt. proteins (85K and 65K) which are not detected in the control virions; the 85K protein may be related to gp85 detected in 3H-glucosamine-labelled ‘interferon’ virions.

Analysis of iodinated proteins of ‘interferon’ virus collected after a short harvest (1 h) revealed fewer virus proteins, principally gp85 and 65K, with only trace amounts of p30 and other structural proteins of the gag gene (data not shown), indicating that the amounts of the high mol. wt. proteins in the ‘interferon’ virions is increased in the rapidly harvested virions.
Differential localization of virus glycoproteins in the virion membranes

It was shown previously (Witte & Weissman, 1974; Witte et al. 1977) that lactoperoxidase treatment of intact MuLV labels predominantly the 78K virus protein which is antigenically related to gp71. To examine how the presence of gp85 in ‘interferon’ virions affects their membrane topology, virions produced in the presence and absence of interferon were
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Fig. 5. Accessibility of virus glycoproteins on the surface of MuLV. Virions produced during a 12 h period in the presence and absence of interferon were labelled by surface iodination, purified on a Sepharose C14B column and disrupted. 125I-labelled proteins were analysed on SDS-PAGE in reducing and non-reducing conditions. Unprecipitated samples (0.1 μg of protein): lane (1), control and lane (2) 'interferon' virions. Iodinated proteins (2 × 10⁶ ct/min) were precipitated with anti-gp71 serum: control (3, 4 and 1), 'interferon' virions (5, 6), in the presence (3, 5) and absence (4, 6) of β-mercaptoethanol. Anti-p30 serum: control (7) and 'interferon' virions (8).

Surface iodination of the control virions revealed the presence of one major surface protein with the mobility of gp71 (Fig. 5, lane 1), when assayed in the presence of mercaptoethanol. To confirm its relationship to gp71, immunoprecipitation was done with the gp71 antiserum. When the precipitate was analysed under reducing conditions, gp71 was detected as a major labelled band (Fig. 5, lane 3) with minor bands in the gp45 and gp32 regions. When analysed under non-reducing conditions (Fig. 5, lane 4), the 125I-labelled gp71 migrated slightly faster and was detected as a double band. In addition, a new 125I-labelled protein with mobility of 90K was seen. It was shown previously that in MuLV a considerable amount of gp71 is linked through disulphide bonding to p15(E) (Pinter & Fleissner, 1977), and these two proteins easily associate during incubation in Triton X-100 (E. Fleissner, personal communication). 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 virus proteins were precipitated with p30 antiserum (Fig. 5, lane 7).
Fig. 6. Densitometer scans of the pulse-chase labelled proteins of MuLV produced in the presence and absence of interferon. JLSV-6 cells persistently infected with MuLV were pulse-labelled with 50 μCi/ml of ³H-labelled amino acid mixture in MEM for 30 min, followed by a 3 h chase with MEM. One set of cultures was treated with interferon (150 units/ml) 24 h before labelling and during the chase. Virus was purified on sucrose gradients and analysed as described in Methods. ---, Interferon virions; ---, control virions. Approximately the same number of ct/min was applied to each gel.

Table 1. Incorporation pattern of ³H-leucine pulse-labelled p30 and gp71 into extracellular virions during chase intervals*

<table>
<thead>
<tr>
<th>p30/gp71 ratio at time intervals:</th>
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<tr>
<td>Control virus</td>
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<tr>
<td>0–3 h</td>
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<td>2.6</td>
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<td>3–6 h</td>
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<tr>
<td>2.7</td>
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<tr>
<td>6–9 h</td>
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<tr>
<td>2.1</td>
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<tr>
<td>Interferon virus</td>
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<tr>
<td>0.9</td>
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<td>1.3</td>
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* Confluent cultures of JLSV-6 cells infected with MuLV were treated with interferon (150 units/ml) for 16 h; after interferon had been removed the cells were pulse-labelled with ³H-leucine (150 μCi/ml) for 30 min in leucine-free medium. The cells were then washed and incubated in medium with interferon for the time indicated. Virus was purified from the collected medium on sucrose gradients and virus proteins were analysed on SDS–PAGE as described in the text. Control virus was harvested from parallel cultures treated in an identical manner in the absence of interferon.

Since foetal calf serum proteins also adhere to the intact virions, it was critical to examine which ¹²⁵I-labelled proteins of the intact virions were precipitated with the FBS antiserum. SDS–PAGE analysis of the precipitate revealed the presence of a labelled band with the mobility of 78K. Normal goat serum did not precipitate any iodinated virion proteins (data not shown).

Surface iodination of the intact 'interferon' virions revealed patterns different from those of the control virions (Fig. 5, lane 2). The standard protein pattern of the iodinated virions disrupted in the presence of mercaptoethanol shows two disperse radioactive bands, 85K and 60K. Analysis of the iodinated proteins after precipitation with gp71 antiserum under both non-reducing and reducing conditions revealed the presence of gp71 (Fig. 5, lanes 5, 6) only. Antiserum to p30 did not precipitate any labelled virus proteins (Fig. 5, lane 8). As in control virions, precipitation with FBS antiserum revealed the presence of a major ¹²⁵I-labelled protein with a mobility of 78K, representing the serum proteins adhering to the
purified virions; no precipitation of \(^{125}\text{I}\)-labelled 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 incorporation of virus proteins into released virions**

To examine whether there is a difference in the rate of incorporation of virus proteins into virions in the presence and absence of interferon, cells grown in the presence and absence of interferon were pulse-labelled with \(^{3}\text{H}\)-amino acids for 30 min, and the radioactivity was chased by incubation in the medium with or without interferon. Virus was purified from the medium collected 3, 6 and 9 h later, and analysed by SDS–PAGE and autoradiography. Quantitative examination of the densitometric scans of the virus protein patterns of the purified virions showed (Fig. 6) that little \(^{3}\text{H}\)-labelled p30 appeared in released virions assembled in the presence of interferon after a chase period of 3 h, whereas this was clearly present in the virions assembled in the absence of interferon. The amounts of \(^{3}\text{H}\)-labelled 55K and 40K were increased in the 'interferon' virions as compared to the controls. In the control virions gp71 was detected as a major \(^{3}\text{H}\)-labelled band in the 70 to 100K regions, while the gp85 was a minor band; virus produced in the presence of interferon contained a \(^{3}\text{H}\)-labelled polydisperse band in the 78 to 95K region.

The observed difference in the packaging of p30 cannot be accounted for by an initial delay in p30 incorporation into the mature virions since the ratio between p30 and gp71 in the virions remained nearly constant during the chase (6 and 9 h) both in the presence and absence of interferon (Table 1). It is also unlikely that the decreased incorporation of p30 into interferon-treated virions reflects interferon-induced inhibition of p30 synthesis, or its processing in the infected cells; no inhibition in p30 synthesis (Friedman & Ramseur, 1974; Pitha *et al.* 1976) or delay in its processing was observed in cells chronically infected with MuLV (unpublished results).

**DISCUSSION**

The biochemical basis for the low infectivity of the MuLV produced in chronically infected cells in the presence of interferon (Pitha *et al.* 1976, 1978) was studied with Rauscher MuLV grown in persistently infected JLSV-6 cells. Our results indicate that interferon treatment leads to the formation of virus particles containing several polypeptides which cannot be detected in the control virions. These novel proteins are precipitable with antiserum to Rauscher MuLV.

Surface iodination of intact virions revealed that in interferon-treated virions both 85K (gp85) and gp71 are the major components of the virion envelope. With control virus, the gp71 (major membrane protein) can associate through a disulphide bond linkage to p15E (Pinter & Fleissner, 1977). In contrast, this gp71–p15E complex was not detected in the virions assembled in the presence of interferon. This lack of gp71–p15E complex formation may be a reason why the 'interferon' virions have lower thermal stability (Pitha *et al.* 1978) and lose gp71 more easily than control virions on sucrose gradient centrifugation or after freezing and thawing (unpublished results). The 85K (gp85) glucosamine-containing and fucose-deficient glycoprotein may be similar to env pr85 (Naso *et al.* 1975, 1976; Van Zaane *et al.* 1975), detected previously in the infected cells.

Analysis of the virions proteins iodinated after virus disruption indicated that the interferon-treated virion contains, in addition to gp85, a number of intermediates of virus structural proteins such as 65K, 55K, 40K and 20K, which were not seen in the control
virus. It is particularly interesting that the novel 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 reactivity with the anti-MuLV serum strongly suggests that these proteins are of virus origin, although the possible cross-reactivity of the MuLV antiserum with cell membrane protein(s) cannot be completely excluded. It has been shown that the carbohydrate portion of gp71 is not essential for reaction with antibody in radioimmunoprecipitation (Bolognesi et al. 1975); the integrity of the protein portion of the molecule is essential. Thus our data may indicate that the polypeptides detected in interferon-treated virions are not completely identical to the uncleaved precursors of env and gag gene products detected in the control infected cells and, therefore, not recognized by the serum whose specificity depends on the primary structure of the protein molecule. Competitive radioimmunoassay with both homologous and heterologous antiserum should make it possible to determine to which extent the structural proteins of the ‘interferon’ and control virions are related.

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 (Yeger et al. 1978). Both previous work by others (Jamjoom et al. 1975; Yoshinaka & Luftig, 1977) and our data indicate 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 final maturation of the core is coupled with further cleavage. Proteolytic activity associated with Rauscher MuLV has been shown to cleave gag pr65 into the lower mol. wt. structural proteins (Yeger et al. 1978). Our data indicate that the cleavage of the precursors of virus proteins is altered in the released interferon virions. Virions harvested during a short time period had significantly more high mol. wt. proteins present than those collected during a longer period of time. Accordingly, the amount of p30 detected in the virions released from interferon-treated cells seems to be time dependent. No difference in the amount of p30 in ‘interferon’ and control virions was detected in 18 h labelling with 3H-amino acid, while the data from the pulse-chase experiments showed that ‘interferon’ virions contained substantially less p30 and more 55K protein than the control virions.

Failure of the processing of env and gag precursors in interferon-treated cells could be due to inhibition of proteolysis either during or after insertion into virions. Interferon may affect a step in protein glycosylation (env pr85) or phosphorylation (pr65) (Pal & Roy-Burman, 1978), and thus change the sensitivity of the virus precursors to subsequent processing (Famulari et al. 1976). It has been shown (Van Zaane et al. 1975) that synthesis, membrane insertion, and glycosylation of proteins may be closely linked; uncoupling of these events by interferon may be responsible for the observed effect. Alternatively, interferon could have a general effect on the cell membrane physiology (Lengyel et al. 1973; Famulari et al. 1976; Chang et al. 1978; Heron et al. 1978). This could change the orientation or insertion of both virus proteins in the plasma membrane, and affect their sensitivity to proteolytic cleavage and the membrane-associated proteases, with the consequent alteration in enzymic activity. The fact that in interferon-treated cells, mature virus particles accumulate on the cell surface (Friedman & Ramsour, 1974; Friedman, 1977; Pitha et al. 1978) is another indication that the block may reside in the assembly-associated proteolytic cleavage required for virus release.

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