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Viral Protein Synthesis in Friend Erythroleukemia Cell Lines

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Viral protein synthesis was studied in two Friend virus-induced erythroleukemia cell lines (Ostertag cell lines FSD1-F4 and B8) by the technique of immunoprecipitation with monospecific antisera to the major envelope glycoprotein gp70 and major core protein p30. One of the cell lines (F4) releases active Friend virus complex to the growth medium, where release of virus from the other cell line (B8) is barely or nondetectable. It was found that in the nonproducer cell line B8, a large-molecular-weight protein of about 65,000 containing p30 antigenic determinants is synthesized, yet no p30 is produced upon prolonged incubation and chase, suggesting that this might be the actual lesion that prevents mature virus production by these cells. In both cell lines, the predominant protein species that is immunoprecipitated with monospecific anti-gp70 serum is a protein of 55,000 to 60,000 daltons that is labeled with glucosamine to a much lesser extent than gp70 and appears to become heterogeneous with time. Large amounts of gp70 can be detected in the cell-free medium, but none of the unstable species of 55,000 to 60,000 molecular weight.

Friend virus-transformed mouse erythroleukemic cell lines have attained widespread use as a model system for the in vitro study of differentiation (5, 16). The Friend cells are morphologically similar to proerythroblasts (19) and are presumably arrested at this stage of differentiation by the virus-induced transforming event; furthermore, many of these cell lines release virus particles into the growth medium (14, 19, 25).

The cell line FSD1/F4 (F4) was established from the spleens of DBA/2 mice infected with NB tropic BALB/c-adapted Friend virus complex by Ostertag et al. (16). While growing in culture, these cells release biologically active Friend virus complex; the lymphatic leukemia helper virus and the replication-defective erythroid cell-transforming spleen focus-forming virus (3). In those studies, the released virus was detected and characterized by electron microscopy and by the XC assay (3). The presence of the spleen focus-forming virus and helper virus was measured by spleen focus formation in DBA/2 and BALB/c mice (N and B type, respectively) after injection of filtered tissue culture supernatants.

A 5-bromodeoxyuridine-resistant and thymidine kinase-negative (TK^-) subclone of line FSD1/F4, clone B8, was found by Ostertag et al. (17) to release 1,000- to 100,000-fold reduced amounts of Friend virus, as compared with the TK^+ parental cell clone F4. Interestingly, morphological studies by electron microscopy revealed that the nonproducer B8 cells contained a great many intracisternal A particles, many more so than the producer F4 cells (14). In spite of these differences, however, both cell clones do share that characteristic property of most Friend cell lines: the capacity to be induced to differentiate upon exposure to dimethyl sulfoxide in the growth medium (3).

We present in this report a characterization of viral protein synthesis in the producer cell line F4 and the nonproducer line B8, arrived at by the application of immunoprecipitation techniques using monospecific antisera to purified viral structural proteins.

MATERIALS AND METHODS

Cell cultures. Friend erythroleukemia cell lines FSD1/F4 (F4) and B8 (3), were generously provided by W. Ostertag, Max-Planck Institut fuer Experimentelle Medizin, Goettingen, West Germany. The cells were grown in suspension cultures in Eagle minimal essential medium (GIBCO, Grand Island, N.Y.) supplemented with 10% fetal calf serum (GIBCO), 2× (the concentration found in Eagle minimal essential medium) glutamine, 1× vitamins, 1× nonessential amino acids, penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (25 μg/ml). The cells were found to be free of mycoplasma contamination as determined by the method of Hayflick (7).

Rauscher leukemia virus-infected mouse 3T3 cells were kindly provided by J. A. Bilello, Albert Einstein College of Medicine, Bronx, N.Y. The cells were propagated in Dulbecco-modified Eagle me-

dium supplemented with 10% fetal calf serum (GIBCO), penicillin (100 U/ml), and streptomycin (100 μg/ml).

Reverse transcriptase assay for virus production. Culture medium was cleared of cells and debris by centrifugation at 2,000 × *g* for 10 min. Virions were concentrated by centrifugation through a 20% glycerol layer in 50 mM Tris (pH 7.8)-0.1 M KCl at 100,000 × *g* for 90 min. Pellets were resuspended as 100-fold concentrates in 50 mM Tris-0.1 M NaCl-1 mM dithiothreitol. Just before assay, the concentrate is diluted, made 0.1% with Triton X-100, and sonicated. The assay is carried out in 0.1-ml-volume reaction mixtures containing 50 mM Tris-hydrochloride buffer (pH 8.0), 50 mM KCl, 8 mM $MnCl_2$, 5 mM dithiothreitol, 0.02 absorbance units of primer template complex at 260 nm, oligo$(dT)_{12-18}$·poly (A), 0.1% Triton X-100, and 5 μCi of [^{3}H]TTP (specific activity, 53 Ci/mM, Schwarz/Mann). Reactions are initiated by the addition of [^{3}H]dTTP and are run for 30 min at 37°C, at the end of which time the reaction is stopped by the addition of 0.5 ml of a 0.1 M sodium pyrophosphate and then 0.5 ml of cold 25% trichloroacetic acid. The mixture is kept at 4°C for 30 min, and then the precipitates are collected on glass-fiber filters (Whatman GF/C) and washed. The filters are dried and then counted in a liquid scintillation counter.

Labeling of cells. Cells in logarithmic growth were spun down at 500 × *g* for 5 min, suspended at a density of 6 × 10^6 cells/ml in 3 ml of serum-free medium buffered with 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) and containing one-twentieth the normal concentration of methionine, and incubated with shaking for 15 min at 37°C. At the end of this preincubation, 200 μCi of [^{35}S]methionine per ml (250 Ci/mmol, Amersham/Searle) was added, and incubation was continued for 45 min. At the end of the labeling period, a portion of the cell suspension was removed for extraction, and the remaining cells were chased for varying periods of time by the addition of a 10,000-fold excess of cold L-methionine. Ten-microliter aliquots of cell suspension were removed at the end of each incubation period for quantitation of [^{35}S]methionine incorporation into protein (12). Cells were washed once with Earle balanced salt solution, and then extracted according to the procedure of Shapiro and August (Biochim. Biophys. Acta, in press), which consists of lysing the cells by the addition of an extraction buffer containing 5 mM Tris-hydrochloride (pH 9.2), 1 mM EDTA, 400 mM KCl, 1% Triton X-100, 1 mM L-1-tosylamido-2-phenylethylchloromethyl ketone, and 1 mM phenylmethylsulfonyl fluoride (PMSF) (protease inhibitors, Sigma Chemical Co., were added to the extraction buffer just before use from a 100 mM stock solution in dimethyl sulfoxide). The suspensions were spun down at 25,000 × *g* for 10 min, and the pellets were reextracted with buffer containing no KCl. The pooled supernatants were dialyzed against TEN buffer and spun at 100,000 × *g* for 60 min before immune precipitation. The dialysis was performed to get rid of some of the Triton X-100 and thereby facilitate immunoprecipitation; no proteolytic degradation of proteins was observed resulting from the dialysis step. Insoluble proteins were removed by the high-speed spin and nonspecific background was reduced.

Labeling of Rauscher virus-infected 3T3 monolayer cells was performed in 75-cm^2 T-flasks using similar conditions as described above for the Friend cells, except that the preincubation was done for 60 min in serum-free medium lacking methionine, and the cells were lysed by scraping them into 2 ml of the extraction buffer.

For 24-h labeling of cells with [^{3}H]glucosamine, 20 μCi of D-[6-^{3}H]glucosamine (Amersham/Searle) was added per ml of a cell culture growing in standard growth media at a density of 8 × 10^5 cells/ml. After the labeling period, the cells were spun down, washed, and extracted, and the cell-free medium was concentrated with a collodion bag (Schleicher and Schuell, Inc., Keene, N.H.) in TEN buffer. Three-hour [^{3}H]glucosamine labeling was performed in cell suspension at a density of 6 × 10^6 cells/ml in standard growth media incubated with shaking in the presence of 100 μCi of D-[6-^{3}H]glucosamine per ml.

Immunoprecipitation. Monospecific goat antisera against purified Rauscher virus core protein p30 and envelope glycoprotein gp 69/71 were the generous gift of T. August and M. Strand, Albert Einstein College of Medicine, Bronx, N.Y. Aliquots, 0.5 ml, of dialyzed and centrifuged cell extracts were incubated with 2 μl of antisera, for a period of 2 h or longer at 4°C. After this period, pig anti-goat immunoglobulin G antiserum was added, and precipitates were allowed to form at 4°C for periods of 6 h or longer, and then washed three times with TEN buffer (20 mM Tris-hydrochloride, pH 7.4, 1 mM EDTA, and 100 mM NaCl), and then once with acetone. The dried pellets were dissolved in 75 μl of electrophoresis sample buffer (10) by incubating at 70°C for 10 min, and then at 100°C for 2 min. Electrophoresis was carried out in 20-cm-long 5 to 20% exponential gradient, sodium dodecyl sulfate (SDS)-polyacrylamide slab gels using the discontinuous Tris-glycine buffer system of Laemmli (10). Autoradiography of dried gels was performed with Kodak Blue medical X-ray film (BB-54). For detection of ^{3}H-labeled proteins, gels were treated by the method of Bonner and Laskey (2) and exposed to Kodak RR Royal X-Omat film at −70°C.

RESULTS

Detection of virus release. Virus production was measured by assaying for reverse transcriptase activity in the culture medium. Aliquots of cell suspension were taken at various times after seeding; cell density was measured, and reverse transcriptase activity in the cell-free medium was determined as outlined under Materials and Methods. As shown in Fig. 1, virus release from producer F4 clone cells is directly related to cell growth. As the cells reach a saturation density of about 2 × 10^6 cells/ml, virus release levels off. Media harvested from growing clone B8 cell cultures,

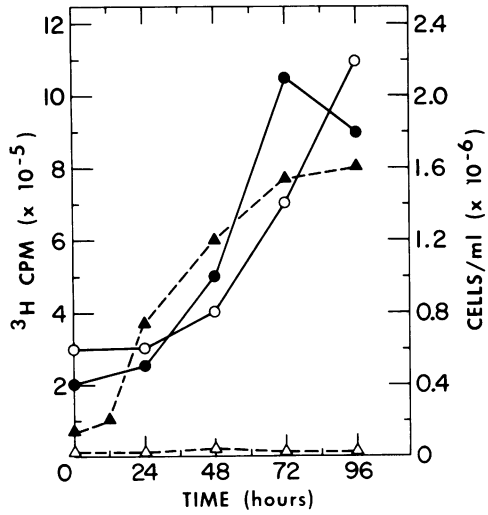


FIG. 1. *Friend leukemia virus production as measured by reverse transcriptase activity in the cell-free medium compared with cell growth. Reverse transcriptase activity released from F4 cells* (▲), *reverse transcriptase activity released from B8 cells* (△). *Viable cells per milliliter in F4 cell culture* (●), *viable cells per milliliter in B8 cell culture* (○).

however, show no detectable reverse transcriptase activity above background levels for this assay, indicating that no significant amounts of virus are released by these cells, as previously reported by Ostertag et al. (17). It may be noted that B8 cells have to be seeded at somewhat higher densities, and grow more slowly than the F4 cells; both cell lines reach similar saturation densities. After 96 h of growth, there is a drop in viable cells per milliliter in the B8 cell culture (not shown), just as in the case of the F4 culture after 72 h (Fig. 1).

Immunoprecipitation of viral proteins. To get a more detailed understanding of virus production by these cells, we undertook to study viral protein synthesis by utilizing immunoprecipitation techniques. Monospecific antisera prepared against purified Rauscher murine leukemia virus proteins were made available to us for this purpose. It has been shown that Rauscher and Friend viruses are very closely related and that their structural proteins share many of the same antigenic determinants (22, 24). Labeled viral proteins were precipitated from Friend cell extracts by these antisera using either the direct or indirect techniques of immunoprecipitation. In using the direct method of immunoprecipitation, similar results were obtained if either Friend or Rauscher virus carrier was added, indicating that Friend virus proteins were bound just as efficiently as Rauscher virus proteins under these conditions. To make the most use of our limited quantities of antisera, the indirect method of immunoprecipitation was utilized throughout these studies.

Findings from several laboratories indicate that the structural proteins of RNA tumor viruses are first synthesized as high-molecular-weight precursors (9, 26, 27), which are subsequently cleaved to yield the mature virion proteins. As shown in Fig. 2, polyacrylamide gel electrophoretic analysis of [^{35}S]methionine-labeled Friend cell extracts, immunoprecipitated with monospecific antisera to the major envelope glycoprotein gp70 and the major core protein p30, reveals several molecular species specifically precipitated with each antiserum. As molecular-weight markers, we have used [^{35}S]methionine-labeled lysates of mouse plasmacytoma (MPC-11) cells infected with vesicular stomatitis virus (13) (Fig. 2a) and BHK-21 cells infected with reovirus (21) (Fig. 2o). Coelectrophoresis of labeled protein standards with unknowns is a more convenient method for comparison purposes on autoradiograms than the commonly used procedure of running unlabeled standards and staining them in the gel. The use of the indirect technique of immunoprecipitation in these studies results in a fairly high background in the gels due to nonspecific adhesion of labeled cellular proteins to the relatively large precipitates. Some very prominent contaminating bands are seen in the region of about 50,000 molecular weight; these are probably actin-type proteins, which are synthesized in large amounts by tissue culture cells; similar observations have been reported by other workers (4, 8). A relatively high degree of radioactive labeling must be achieved in these cells to detect viral proteins, for they only constitute approximately 0.1% of total cellular protein synthesis (unpublished observation).

Figures 2c, d, and e show [^{35}S]methionine-labeled F4 cell extracts precipitated with anti-gp70 serum. In all three channels, 45-min labeling (c), 60-min chase (d), and 120-min chase (e), it can be seen that most of the gp70-specific label is present in a broad band, with apparent molecular weight between 55,000 and 60,000, which seems to migrate faster with increasing chase time. There is a band in the expected region of about 70,000 daltons, and a possible precursor of 80,000-molecular-weight which disappears after a 60-min chase. A low-molecular-weight protein of less than 20,000 is also specifically precipitated in all three extracts. Figures 2f, g, and h show aliquots of the same extracts precipitated with anti-p30 serum. As has been reported for Rauscher virus-producing cells (9, 26), p30-specific label is initially incorporated

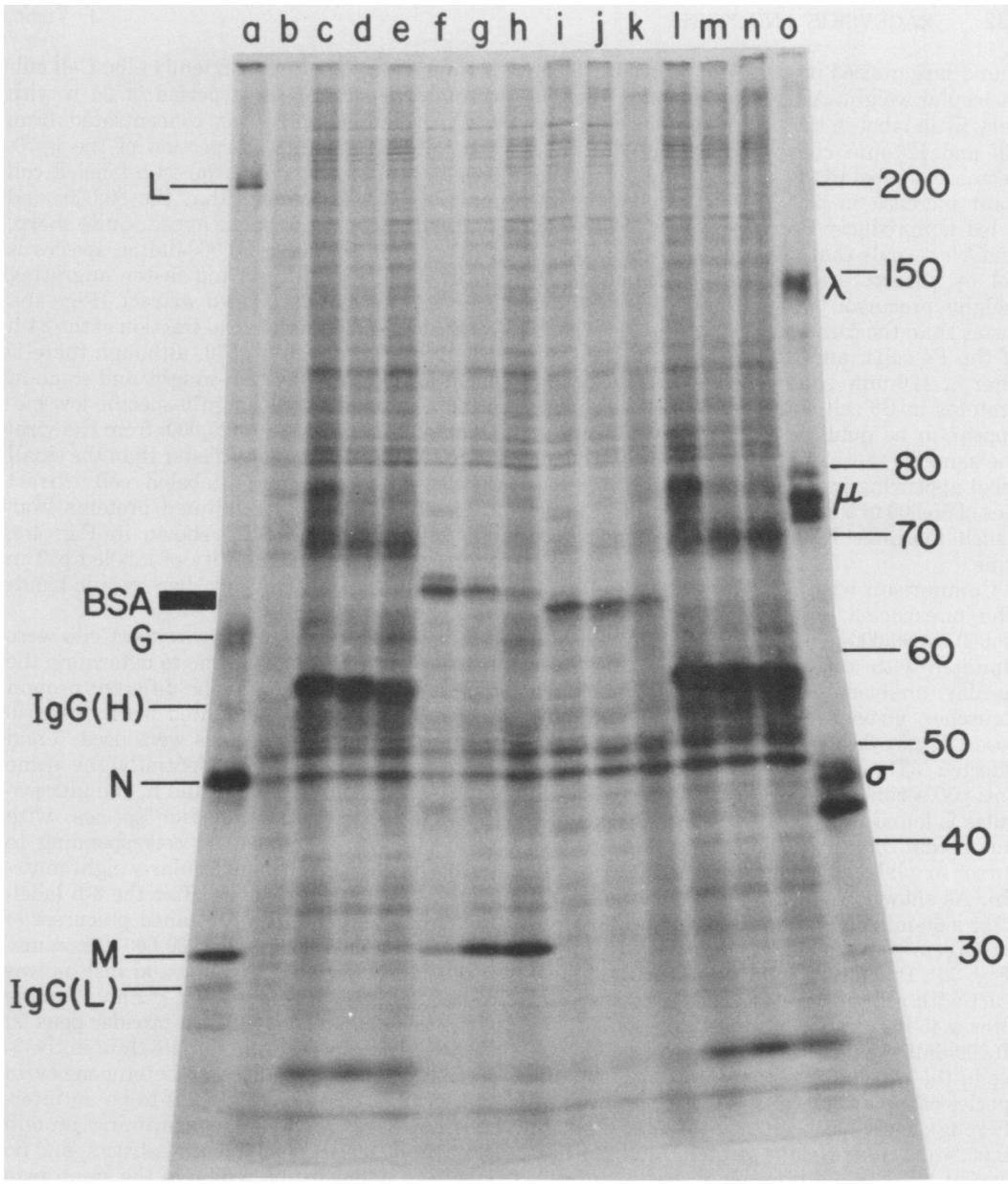


FIG. 2. *Autoradiograph of SDS-gel electrophoresis of immunoprecipitates from Friend cell extracts pulse-labeled with [^{35}S]methionine. A scale of approximate molecular weights in kilodaltons has been included alongside the gel for orientation purposes. The approximate position of migration of unlabeled bovine serum albumin (BSA) (molecular weight, 68,000) has also been drawn in. (a) Molecular-weight markers of [^{35}S]methionine-labeled lysate of mouse plasmacytoma (MPC-11) cells infected with vesicular stomatitis virus (VSV), showing the following proteins with corresponding molecular weights: L, 195,000; G, 66,000; MPC-11 immunoglobulin G (IgG) (H), 53,000; N, 48,000; M, 29,000; and MPC-11 IgG (L), 22,000. (b) Control 45-min [^{35}S]methionine-labeled, F4 cell extract precipitated with nonimmune goat serum. (c) A 45-min [^{35}S]methionine-labeled F4 cell extract immunoprecipitated with anti-gp70 serum. (d) Same as (c), but chased 60 min with 10,000× excess cold L-methionine. (e) Same as (c), but chased 120 min. (f) Same as (c), except precipitated with anti-p30 serum. (g) Same as (f), but chased 60 min. (h) Same as (f), but chased 120 min. (i) A 45-min [^{35}S]methionine-labeled B8 cell extract immunoprecipitated with anti-p30 serum. (j) Same as (i), but chased 60 min. (k) Same as (i), but chased 120 min. (l) Same as (i), except immunoprecipitated with anti-gp70 serum. (m) Same as (l), but chased 60 min. (n) Same as (l), but chased 120 min. (o) Molecular-weight markers of [^{35}S]methionine-labeled lysate of BHK-21 cells infected with reovirus, showing the three protein groups with the following molecular weight ranges: λ, 143,000 to 153,000; μ, 72,000 to 79,000; σ, 43,000 to 54,000.*

into a larger-sized precursor of 65,000 to 70,000 molecular weight. After a 45-min pulse, there is very little label in the p30 region, but after the 60- and 120-min chase periods we can see a decrease of label in the precursor and a concomitant increase in p30. An examination of labeled nonproducer B8 cell extracts (Fig. 2i, j, and k), reveals that, similarly, p30-specific label is incorporated into a high-molecular-weight precursor which, however, migrates faster than the corresponding precursor protein of the F4 cells, and that no p30 appears even after a 120-min chase. Labeled gp70-specific proteins in B8 cell extracts (Fig. 2l, m, and n) appear to be qualitatively and quantitatively the same as those of F4 cells, with most of the label appearing in an apparently unstable species of 55,000 to 60,000 initial molecular weight, which migrates faster with increasing chase time.

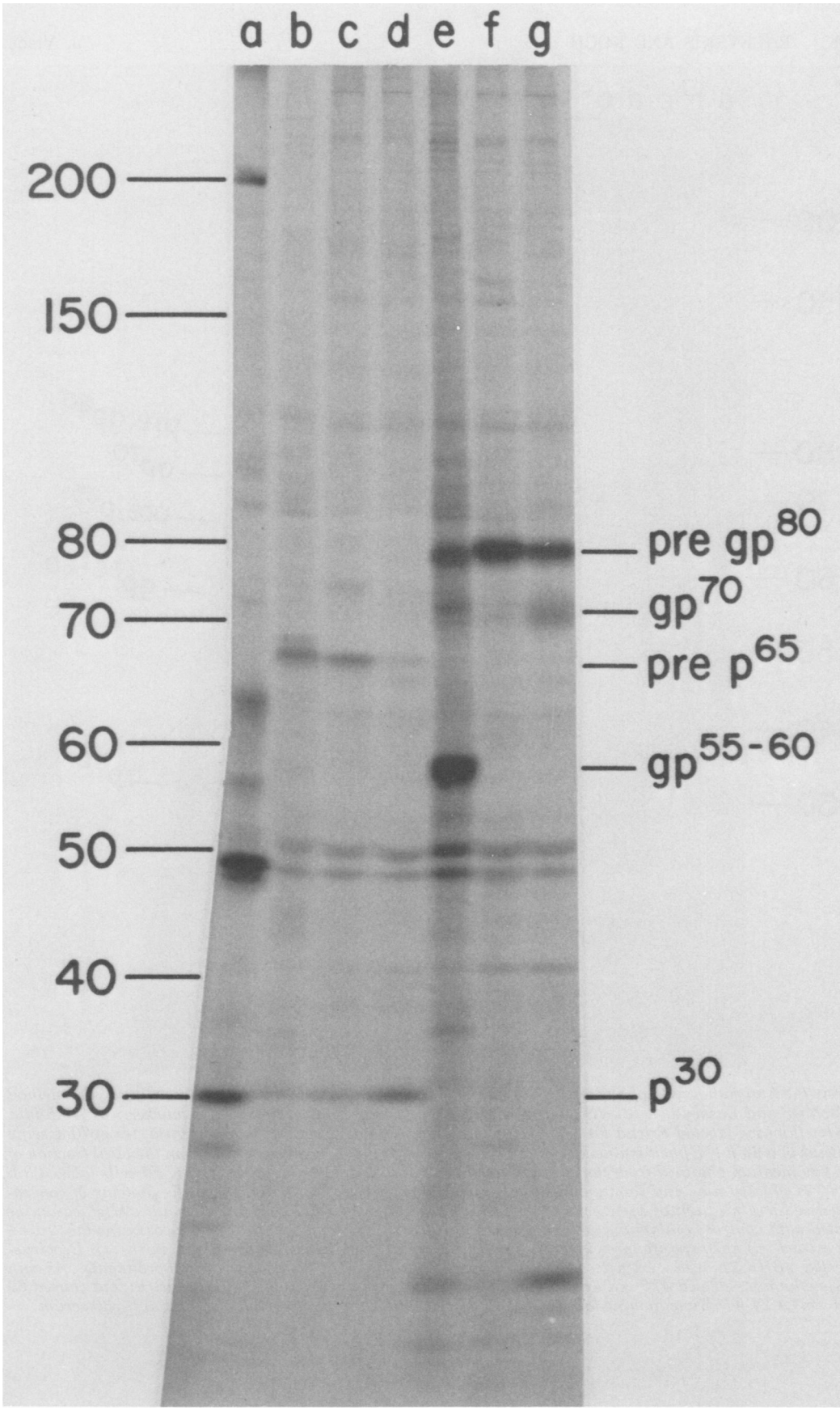
Comparison with Rauscher virus proteins. The unexpected observation of the unstable 55,000- to 60,000-molecular-weight protein precipitated with anti-gp70 serum necessitated a similar analysis of the better-characterized Rauscher virus system for comparison purposes. Figure 3c shows that, in Rauscher virus-infected 3T3 cell extracts precipitated with anti-p30 serum, most of the label after a 45-min pulse is found in a 65,000- to 70,000-dalton precursor that comigrates with the analogous precursor in a labeled Friend cell (F4) extract (Fig. 3b). As shown previously for the Friend cells, after a 60-min chase, most of the label is lost in the precursor region and has increased in p30 (Fig. 3d). Precipitation of the Rauscher cell extract with anti-gp70 serum (Fig. 3f) shows that after a 45-min pulse, most of the label is in an 80,000-dalton protein and that none is detectable in the region corresponding to the unstable species of the Friend cells. After a 60-min chase (Fig. 3g), there has been a loss of label from the 80,000-molecular-weight protein and appearance of label in a diffuse band that is presumably gp70. As in the case of the gp70-specific proteins of the Friend cells, there is also precipitation of a small-molecular-weight protein (<20,000) in the Rauscher virus-producing cells. The 80,000-dalton precursor glycoprotein seems to be more stable in the Rauscher virus cells than in the Friend cells, for in the latter the corresponding protein is no longer detectable after a 60-min chase.

Long-term labeling of friend cells. Cell cultures were labeled for a period of 24 h with [^{35}S]methionine and virus concentrated from the cell-free medium. Inspection of the gp70-specific protein bands in the 24-h-labeled cell extract (Fig. 4c) reveals that the 80,000- and 70,000-dalton protein bands appear quite sharp, whereas the 55,000- to 60,000-dalton species is noticeably more diffuse and faster migrating than in the 45-min-labeled extract (Fig. 4b). Most of the label in the viral fraction of the 24-h labeling (Fig. 4d) is in gp70, although there is some at 80,000 molecular weight and some at 60,000. Interestingly, the gp70-specific low-molecular-weight protein (<20,000) from the viral fraction (Fig. 4d) migrates faster than the small species from the 45-min-labeled cell extract (Fig. 4b). Anti-p30 precipitated proteins from the 24-h-labeled cells are shown in Fig. 4m; there was a similar quantity of labeled p30 in the viral fraction, with no other specific bands visible (not shown).

Labeling of viral glycoproteins. Cells were labeled with [^{3}H]glucosamine to determine the degree of glycosylation in the different protein species specifically precipitated with anti-gp70 serum. Two labeling periods were used, 3 and 24 h, and both showed essentially the same profiles. As seen in Fig. 4e and h, the glucosamine is incorporated into four species, with most of it in a broad band corresponding to gp70, some in a higher-molecular-weight material that is best discernible after the 3-h labeling (Fig. 4e) and is the presumed precursor of gp70. The unstable 55,000- to 60,000-dalton material is glycosylated as well, and just as was seen after 24-h labeling with [^{35}S]methionine (Fig. 4c), it travels as a much broader peak in the 24-h [^{3}H]glucosamine sample than in the 3-h sample, indicating greater heterogeneity in the former. There also appears to be an intermediate-sized glucosamine-containing protein of about 65,000 daltons in both extracts, and no label is evident in the region of the small peptide of less than 20,000 daltons that is observed in [^{35}S]methionine-labeled extracts precipitated with anti-gp70 serum.

The concentrated supernatant of the culture medium was immunoprecipitated after the labeling periods, and Fig. 4f and i reveal that there is a large amount of [^{3}H]glucosamine-labeled gp70 in the culture medium, presumably released as part of the budding virions.

FIG. 3. *(a) VSV-infected MPC-11 cell extract molecular-weight marker. (b) p30-specific proteins of a 45-min [^{35}S]methionine-pulsed Friend cell (F4) extract. (c) p30-specific proteins of a 45-min [^{35}S]methionine-pulsed Rauscher virus-producing 3T3 cell extract. (d) Same as (c) but chased 60 min. (e) Same as (b) except immunoprecipitated with anti-gp70 serum. (f) Same as (c) except immunoprecipitated with anti-gp70 serum. (g) Same as (f) but chased 60 min.*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p30

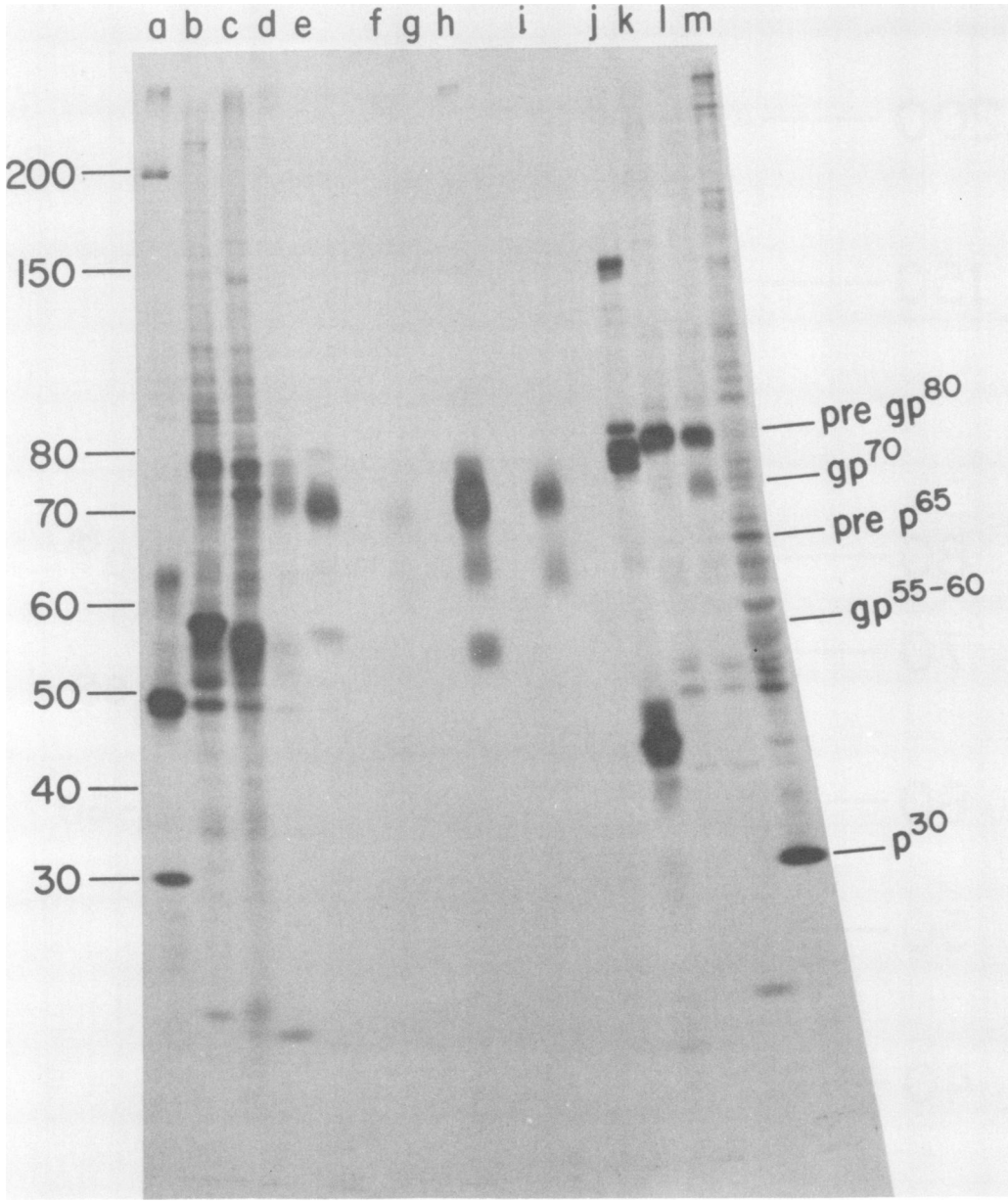

FIG. 4. *Autoradiograph of a dimethyl sulfoxide-, 2,5-diphenyloxazole-treated gel according to the method of Bonner and Laskey (2). (a) VSV-infected MPC-11 cell extract molecular-weight marker. (b) A 45-min [^{35}S]methionine-labeled Friend F4 cell extract immunoprecipitated with anti-gp70 serum. (c) gp70-specific proteins of a 24-h [^{35}S]methionine-labeled F4 cell extract. (d) gp70-specific proteins from the viral fraction of cell-free medium obtained from the 24-h [^{35}S]methionine-labeled F4 cells. (e) Extract of F4 cells labeled 3 h with [^{3}H]glucosamine and immunoprecipitated with anti-gp70 serum. (f) gp70-specific proteins of concentrated cell-free medium of same cells as in (e). (g) Extract of F4 cells labeled 24 h with [^{3}H]glucosamine treated with control nonimmune goat serum. (h) gp70-specific proteins of 24 h [^{3}H]glucosamine-labeled F4 cell extract. (i) gp70-specific proteins of concentrated cell-free medium of same cells as in (h). (j) Reovirus-infected BHK-21 cell extract molecular-weight marker. (k) Rauscher virus-producing 45-min [^{35}S]methionine-labeled 3T3 cell extracts precipitated with anti-gp70 serum. (l) Same as (k), but chased 60 min. (m) A 24-h [^{35}S]methionine-labeled Friend F4 cell extract immunoprecipitated with anti-p30 serum.*

Most striking, however, is the fact that none of the unstable 55,000- to 60,000-dalton species is observed in the media, whereas some of the intermediate-sized material of 65,000 molecular weight is present.

DISCUSSION

The observation that the nonproducer B8 cells synthesize a p30-specific precursor protein that is not eventually processed to form p30 suggests that this might be the actual lesion that prevents mature virus production by these cells. A similar observation has been reported by Stephenson et al. (23) in the case of mutants of Rauscher virus, where the restriction to replication was shown to be associated with the intracellular accumulation at the restrictive temperature of a 70,000-dalton precursor containing antigenic reactivities of three viral structural proteins: p30, p15, and p12. Stephenson et al. (23) also reported that the precursor was rapidly cleaved when infected cells were shifted down to the permissive temperature in the absence of further protein synthesis. They speculated that the temperature sensitivity may be a property of the precursor, such as a reversible conformational defect. In the case of the B8 cells, it is clear that the high-molecular-weight precursor itself is altered, since it migrates faster than the corresponding molecule in the producer F4 cells, suggesting a deletion type of defect or an improper cleavage in an earlier maturation step. Restriction to replication that is caused by a block in precursor cleavage has also been shown in avian virus-transformed hamster cells (4). In that system, however, Eisenman et al. (4) suggested that, on the basis of their observations, the most likely explanation for the block was that a specific protease was either present or virus inducible in avian cells and absent from the avian virus-transformed hamster cells.

Of possible relevance is the observation by Ostertag et al. (14) that, even though the B8 cells produce barely detectable amounts of type C particles, they do display many intracisternal virus-like A particles when viewed under the electron microscope. Evidence presented in a recent report by Robertson et al. (18) indicates that the intracisternal A particles and extracellular type C particles produced by a mouse myeloma cell line are closely related, and is suggestive of a precursor-product relationship between the two types of particles. On the basis of these reports, it is tempting to speculate that the A particles observed in B8 cells are immature virions arrested at that stage of maturation by the uncleaved precursor protein.

In the two Friend cell lines studied, the most abundant protein that is precipitated with anti-gp70 serum is a species of 55,000 to 60,000 molecular weight, which migrates faster and becomes more heterogeneous with increasing incubation time. It is not an artifact resulting from some experimental procedure used in these studies, since an identical analysis of a Rauscher virus-producing cell line reveals no analogous peptide. This protein incorporates glucosamine to a much lesser extent than gp70 and does not seem to be released from the cell. Some workers have previously reported the presence of a very minor glycoprotein component in Friend virus of approximate molecular weight of 60,000 or 45,000 (1, 11). Whether this is the same species that was observed in our study cannot be determined from the data given in those reports. Of possible interest is the observation by Bolognesi et al. (1) that treatment of intact Friend virus preparations with glycosidases removed about 80% of the glucosamine-labeled material from gp70, and that the remaining material migrated as a broader peak to a position of about 60,000 to 65,000 molecular weight. In all studies dealing with characterization of glycoproteins, it must be kept in mind that glycoproteins display an anomalous migration on SDS-polyacrylamide gel electrophoresis because of a decreased binding of SDS to the oligosaccharide side chains relative to the polypeptide backbone (20). It is possible that the protein moiety of the faster-migrating species in these cells is identical to that of gp70; the large difference in migration may be accounted for by its lesser degree of glycosylation, which would result in two additive effects: a higher relative binding of SDS and a smaller actual molecular size. The broadening of the peak, loss of label seen after extended incubation periods, and absence from the culture medium is indicative of intracellular degradation of the material, which suggests that the complete carbohydrate side chains are necessary to protect the viral glycoprotein from degradation.

All three cell lines analyzed in this study, Friend leukemia cell lines F4 and B8 and Rauscher virus-infected 3T3 cells, showed the presence of a small-molecular-weight protein of less than 20,000 being specifically precipitated with monospecific anti-gp70 serum. A similar, but faster migrating, protein was also present in virus pelleted from cell-free media of an F4 cell line culture labeled for 24 h. Ongoing studies have revealed that these proteins migrate slower than p12 on an SDS gel and do not cross-react with antisera to either p12 or p15 (Racev-

skis and Koch, unpublished observation). The fact that these peptides share antigenic determinants with gp70 suggests that they might be breakdown products of gp70. In support of our observation is a very recent report by Naso et al. (12), which describes the presence of two small nonglycosylated proteins (termed p15E and p12E) in Rauscher virus-infected JLS-V16 cells, which share tryptic peptide sequences with the major glycoprotein and its precursor. Furthermore, Naso et al. suggest a precursor-product relationship between p15E and p12E, which is in accord with our observation that the smaller species are found in the long-term-labeling virus preparation. More in-depth studies will be required to elucidate the role of these peptides in the viral life cycle.

In addition to the two cell lines described in this report, we have analyzed four more Friend erythroleukemia cell lines established by W. Ostertag, and one line established by C. Friend, CL-745, and have observed in all of them the same characteristic pattern of viral proteins, with the prominent 55,000- to 60,000-dalton species being precipitated with anti-gp70 serum (Racevskis and Koch, unpublished observation). Whether this pattern of viral protein synthesis is characteristic of all Friend cell lines, and is an integral part of their unique properties, will have to await further study.

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