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Biochemical and Immunological Analysis of Human Immunodeficiency Virus *gag* Gene Products p17 and p24

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Human immunodeficiency virus (HIV) p24 was purified to homogeneity and subjected to NH_2-terminal sequencing. The sequence determined perfectly corresponded to the amino acid sequence predicted from the nucleotide sequence of a middle portion of the HIV first open frame: the *gag* gene. Edman degradation of purified HIV p17 revealed instead a blocked NH_2 terminus. Hybridomas secreting monoclonal antibodies to p24 and p17 were developed and used to immunologically characterize these two HIV *gag* gene products. They identified two *gag* precursor polyproteins in the cytoplasm of HIV-infected cells: $Pr53^{gag}$, which corresponds to the primary translational product, and $Pr39^{gag}$, which corresponds to an intermediate product of cleavage of $Pr53^{gag}$. These monoclonal antibodies allowed us also to study posttranslational modification of HIV p24 and p17. p24 was found to be phosphorylated, which is a very unusual feature for a major retroviral core protein. p17 was found to be myristylated, as are all NH_2-terminal *gag* proteins of the known human retroviruses.

Human immunodeficiency virus (HIV), is the exogenous retrovirus identified as the primary etiologic agent of the acquired immune deficiency syndrome (AIDS) and related diseases (5, 10, 28). Various strains of the virus have been isolated in several laboratories (human T-cell lymphotropic virus type III [HTLV-III], lymphadenopathy-associated virus, and AIDS-related virus) (5, 16, 20). The proviral genes corresponding to many of these isolates have been cloned, and their complete primary nucleotide sequences have been determined (21, 25, 38). The overall genomic structure of the provirus resembles that of all replication-competent retroviruses, since three essential genes were identified. These include the *gag* gene, coding for the major nonglycosylated viral structural proteins, the *pol* gene, coding for the RNA-dependent DNA polymerase (reverse transcriptase), and the *env* gene, coding for components of the viral envelope. In addition, at least four functional nonstructural genes have been identified: *sor* (15), *tat*-III (4, 31), 3'*orf* (3), and *art/trs* (9, 30). Most of the antigens encoded by these open reading frames are specifically recognized by antibodies present in serum from many patients with AIDS and AIDS-related complex and asymptomatic people infected with HIV. Antibodies in most virus-infected people identify antigens of 120, 66, 51, 41, 31, 24, and 17 kilodaltons when tested in immunoblot assays with lysates of $HTLV\text{-}III_B$. Of these antigens, p120 and p41 have been identified as *env* gene products and are glycosylated (1, 37). p51/66 and p31 have been shown to be products of the *pol* gene of HIV and represent, respectively, the reverse transcriptase (36) and endonuclease (2, 32) of this virus. Several lines of evidence suggest that p24 and p17 are the *gag* gene products. First, the nucleotide sequence data indicated that the first long open reading frame could encode the *gag* precursor (21). Amino acid similarities to the *gag* gene products of HTLV-I were detected in the middle of this open reading frame. Moreover, a great accumulation of p24 is found by Coomassie blue staining of purified viral preparations separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), and this parallels the findings with HTLV-I and HTLV-II (28). A cleavage of the putative *gag* gene product precursor to yield p24 would produce an amino-terminal protein similar in length to the animo-terminal protein of HTLV-I. p17 probably corresponds to this product.

Since interpretation of serological assays currently in use to diagnose HIV infection requires a clear understanding of the viral antigens, a definitive assignment of p17 and p24 was needed. In this paper we report on the direct characterization of these proteins as a result of determination of amino acid sequences of $HTLV\text{-}III_B$ p24 and p17 and of development of monoclonal antibodies recognizing these two proteins. These antibodies were instrumental in identifying the cellular precursor molecule and in studying posttranslational modification of p24 and p17.

MATERIALS AND METHODS

Cells. $H9/HTLV\text{-}III_B$ is a long-term-cultured human T cell line infected with and producing $HTLV\text{-}III_B$ (20). These cells were grown in RPMI 1640 containing 100 μg of penicillin per ml, 50 μg of streptomycin per ml, 2 mM L-glutamine, and 10% heat-inactivated fetal calf serum in a humidified incubator at 37°C and containing 5% CO_2.

Virus. $HTLV\text{-}III_B$ was grown in tissue culture of $H9/HTLV\text{-}III_B$ cells as described previously (20). Culture media were concentrated, and the virus was purified by banding in a sucrose density gradient twice. Clarified supernatant from disrupted virus was used as the source of antigen in immunological assays.

Preparation of monoclonal antibody. BALB/c mice (Charles River Breeding Laboratories, Inc.) were immunized by successive intraperitoneal inoculations of detergent lysates of density gradient-purified $HTLV\text{-}III_B$ (100 μg) emulsified in complete Freund adjuvant for the first inoculation and in incomplete Freund adjuvant for the following four boosters, given 1 week apart. Three days following a final intraperitoneal booster with disrupted virus in phos-

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phate-buffered saline (PBS), splenic lymphocytes were fused with the NS-1 mouse myeloma cell line. The fusion procedure, cell culturing, determination of immunoglobulin subclass secretion, and cloning of hybridoma lines were very similar to procedures previously described in the literature (22). Mouse ascitic fluid containing monoclonal antibodies was prepared as described previously (22).

Enzyme-linked immunosorbent assay. Supernatant fluids of hybrids obtained were screened by enzyme-linked immunosorbent assay with detergent-disrupted HTLV-III_B as antigen. Wells of 96-well plastic trays were coated overnight with a lysate of density-banded HTLV-III_B at 0.5 μg of protein per well in 100 μl of 50 mM sodium bicarbonate buffer (pH 9.6). The wells were then washed once with distilled water and incubated overnight at 4°C with 100 μl of each individual hybrid cell supernatant fluid to be tested. They were then washed three times with 0.05% Tween 20 in PBS and incubated for 1 h at room temperature with affinity-purified, peroxidase-labeled goat antibody to mouse immunoglobulin G (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) at a dilution of 1:1,000 in PBS containing 1% normal goat serum. The wells were washed four times with 0.05% Tween 20 in PBS and four times with PBS and were treated with 100 μl of the substrate mixture containing 0.05% *o*-phenylenediamine and 0.005% hydrogen peroxide in phosphate-citrate buffer (pH 5.0). The reaction was stopped by the addition of 50 μl of 2 M H_2SO_4, and the color yield was measured at 492 nm with a spectrophotometer. An A_{492} of 10 times that of the negative control was taken as positive.

Western immunoblot. Lysates of HTLV-III_B were fractionated by SDS-PAGE. The proteins were electrophoretically transferred to nitrocellulose sheets as described by Towbin et al. (34). The sheets were incubated at 37°C for 3 h in 5% bovine serum albumin–10 mM Tris hydrochloride (pH 7.4)–0.9% NaCl and cut into strips. Each strip was incubated for 2 h at room temperature and overnight at 4°C with a 1:1,000 dilution of conventional control serum or a 1:10 dilution of spent supernatant fluid from hybrids. The incubation buffer (buffer I) consisted of 20 mM Tris hydrochloride, 1 mM phenylmethylsulfonyl fluoride (pH 7.4), 1 mM EDTA, 0.2 M NaCl, 0.3% Triton X-100, and 2 mg of bovine serum albumin per ml. After incubation, the strips were washed three times with 10 mM Tris hydrochloride (pH 7.4)–0.9% NaCl–0.5% Triton X-100–0.3% sodium deoxycholate–1 mM EDTA. The strips were incubated at room temperature with buffer I containing 4% normal goat serum. ^{125}I-labeled goat antibody (5×10^5 cpm/ml) to mouse immunoglobulin G, heavy- and light-chain specific (Cappel Laboratories, Cochranville, Pa.), was added to the incubation mixture for 30 min. The strips were washed three times as before, briefly dried, and exposed to X-ray film.

Radiolabeling of cells. Cells to be labeled with [^{35}S]cysteine, [^3H]lysine, $^{32}P_i$, or [^3H]myristic acid were suspended for 1 h at 37°C at 10^6 cells per ml in cysteine-free, lysine-free, phosphate-free, or normal RPMI 1640, respectively, containing 10% dialyzed serum, 1% glutamine, and 1% penicillin–streptomycin. [^{35}S]cysteine and [^3H]lysine were then added to a final concentration of 100 μCi/ml, and the cells were incubated for 18 h at 37°C. Alternatively, $^{32}P_i$ or [^3H]myristic acid was added to a final concentration of 1 mCi/ml, and the cells were incubated for 3 h at 37°C. After labeling, cells were washed once in ice-cold PBS, pelleted and suspended in PBS-TDS (10 mM sodium phosphate [pH 7.2] containing 0.5% NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% SDS).

RIP and SDS-PAGE analysis. Labeled cells in PBS-TDS were disrupted at 4°C by repeated aspiration through a 25-gauge needle. Portions of the lysates to be immunoprecipitated with human sera were absorbed for 3 h at room temperature with protein A-Sepharose (PAS) and equal portions of normal sera. Portions of the lysates to be immunoprecipitated with monoclonal antibodies were instead preabsorbed with PAS bound to rabbit antiserum to mouse κ light chain (K-PAS). The lysates were then clarified by centrifugation. Radioimmunoprecipitation (RIP) analysis was performed by adding either 1.0 μl of ascites fluid and 0.2 ml of a 10% suspension of K-PAS or 10 μl of human sera and 0.2 ml of a 10% suspension of PAS to 1 ml of labeled and clarified extract. The samples were incubated for 18 h at 4°C. Immunoprecipitates were collected by centrifugation at 2,000 × *g* for 10 min, washed repeatedly in PBS-TDS, suspended in 50 μl of 0.65 M Tris hydrochloride (pH 6.7)–1% SDS–10% glycerol–2.5% 2-mercaptoethanol–0.1% bromophenol blue, heated for 2 min at 90°C, and analyzed by SDS-PAGE.

Purification of p24. Density-banded HTLV-III_B was lysed with 0.5% Nonidet P-40 in 0.8 M NaCl and 0.1 mM phenylmethylsulfonyl fluoride by Dounce homogenization. The lysate was clarified by centrifugation at 100,000 × *g* for 2 h. The clarified supernatant was filtered and subjected to gel filtration on a high-performance liquid chromatography (HPLC) column (TOYOSODA TSKG2000SWG) in 10 mM sodium phosphate (pH 7.0) containing 0.1% SDS. Fractions containing the peak of p24 as judged by immunoblots with sera from HIV-infected individuals were pooled, acidified with trifluoroacetic acid to pH 2, filtered, and subjected to reverse-phase HPLC on a μBondapak C_{18} column (Waters Associates, Inc., Milford, Mass.). The column was developed with a linear 0 to 50% aqueous acetonitrile gradient containing 0.1% trifluoroacetic acid as described previously (19), and the protein recovered under the major peak was used for immunological and chemical analysis.

Purification of p17. p17 was obtained by reverse-phase HPLC of disrupted virus as described in detail previously (13). The solvents and column used were the same as for the p24 purification. A portion of the protein obtained was taken for amino acid analysis.

Sequence analysis. Aliquots of the protein in the acetonitrile-water solution were applied to the spinning cup of a liquid-phase sequenator (Beckman Instruments, Inc., Fullerton, Calif.) and subjected to semi-automated Edman degradation as described previously (8). The phenylthiohydantoin amino acid of each cycle was identified by reverse-phase HPLC on a phenylalkyl column as described previously (11).

RESULTS

Purification and NH_2-terminal amino acid sequence of HIV p24. The major internal protein, p24, of HIV was purified from concentrated preparations of density-banded HTLV-III_B by previously described methods (7, 19, 26). The purity of p24 was confirmed by HPLC where the protein eluted as a single peak (Fig. 1) and by SDS-PAGE analysis (data not shown). The identity of p24 was confirmed by immunoblot of protein-containing fractions with serum from an AIDS patient as first antibody. The only band in the autoradiogram recognized by the human serum corresponds to p24 (Fig. 1). To determine the NH_2-terminal amino acid sequence of HTLV-III_B p24, we degraded 2 nmol of protein in a single microsequence analysis. Unambiguous identification of phenylthiohydantoin derivatives of amino acids was possible up to residue 22. The NH_2-terminal amino acid sequence of

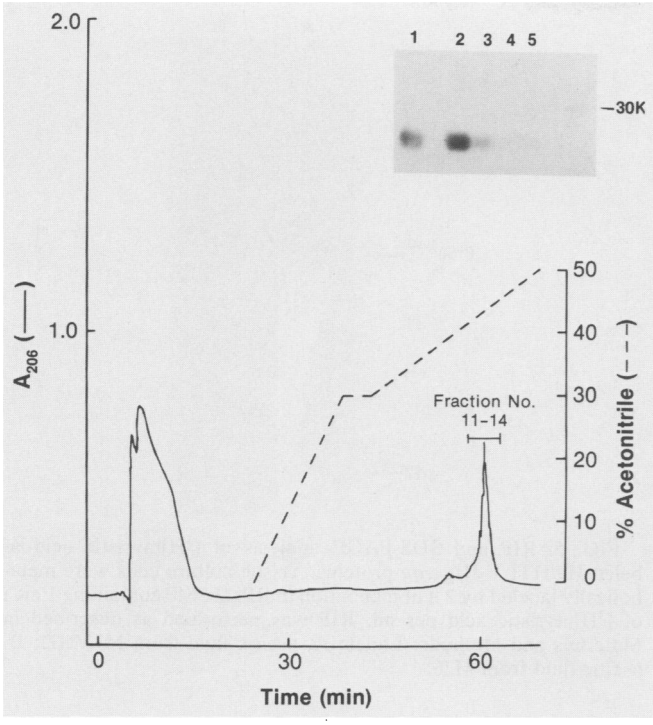

FIG. 1. HPLC profile of HTLV-III$_B$ p24. Purified p24 dissolved in 0.01 M phosphate buffer (pH 7.0)–0.1% SDS was acidified with trifluoracetic acid to pH2 and applied to a μBondapak C_{18} column. The column was developed with an (0 to 50%) acetonitrile gradient. Portions from every fraction of the peak were run on SDS-PAGE, blotted to nitrocellulose sheets, and reacted with a human antiserum from an individual infected with HIV. The insert shows the results of this analysis. Lane 1 represents the load, and lanes 2 through 5 represent fractions 11 through 14. The molecular weight marker shown is carbonic anhydrase.

the p24 is given in Fig. 2. The sequence determined is a perfect match of the amino acid sequence for p24 predicted from the nucleotide sequence of the HTLV-III$_B$ provirus clones BH10 and BH5. The NH_2-terminal amino acid is proline, which is conserved in the homologous core proteins of all retroviruses.

Purification and NH_2-terminal amino acid sequence of HIV p17. The purity and identity of HIV p17 were confirmed by the same approach as for p24 as described above. The protein eluted as a single peak, and p17 was the only band recognized by the positive human serum in immunoblots (data not shown). To determine its NH_2-terminal amino acid sequence, we subjected p17 to microsequence analysis. No phenylthiohydantoin derivatives of amino acids were observed in the first 10 degradative cycles. This result showed HIV p17 to be inaccessible to Edman degradation and suggested an NH_2-terminal modification.

Development of monoclonal antibodies to HIV p24 and p17. To select hybridomas secreting monoclonal antibodies against HTLV-III p24 and p17, we screened supernatant fluids from growing hybrids by enzyme-linked immunosorbent assay with disrupted HTLV-III$_B$ as antigen. Supernatants that scored positive in this assay were further tested by immunoblots at first against HTLV-III$_B$ viral lysates and then against lysates from both HTLV-III$_B$-infected and uninfected H9 cells. Hybrids scoring positive on viral lysate and on virus-infected H9 cell lysate, but negative on uninfected H9 lysate were cloned by limiting dilution. Among all the virus specific hybridomas, we selected the ones secreting antibodies to p24 and p17 by molecular weight determination of the antigens identified and by comparison of their reactivity with that obtained with HIV antibody-positive human sera. A further selection involved the ability of these hybridomas to precipitate the corresponding antigen. A summary of specificities and characteristics of the two hybrid clones of choice is given in Table 1. These antibodies were also tested against HTLV-I and HTLV-II lysates, and no cross-reactive epitopes could be observed, identifying the determinants recognized as type specific.

	1	5	10	15	20
p24	P	I	V	Q	N	I	Q	G	Q	M	V	H	Q	A	I	S	P	R	T	L	N	A
p17	Blocked																					

FIG. 2. NH_2-terminal amino acid sequence of HTLV-III$_B$ p24 and p17.

***gag* precursors.** To identify the *gag* precursor polyprotein in the cytoplasm of infected cells, we analyzed lysates of HTLV-III$_B$-producing cells by RIP and SDS-PAGE. Log-phase cultures of H9/HTLV-III$_B$ were labeled with [^{35}S] cysteine overnight. The cells were lysed with detergents, and the clarified extracts were treated overnight with ascitic fluid from two independent anti-p24 hybridomas and two independent anti-p17 hybridomas. The precipitates were collected and analyzed by SDS-PAGE. Results obtained are shown in Fig. 3. Although the anti-p24 and anti-p17 monoclonal antibodies precipitated the respective proteins, both also precipitated a common 53-kilodalton protein. Therefore, the same antigenic determinants recognized on the cleaved products are present on the p53 molecule. This result identifies the cellular p53 as the precursor of HIV *gag* proteins. RIP and SDS-PAGE analysis also identified a possible intermediate product of cleavage of the p53 *gag* precursor or an alternate precursor present in the cytoplasm of [^{3}H]lysine-labeled HTLV-III$_B$-infected H9 cells. A protein of 39,000 daltons was precipitated by HIV antibody-positive human sera and by both anti-p24 and anti-p17 monoclonal antibodies (Fig. 4). p39 could also be detected if [^{35}S]cysteine instead of [^{3}H]lysine was used as a label, but required longer exposure of the autoradiogram. The monoclonal antibody to HIV gp41 (M25) did not precipitate p39, but efficiently precipitated the *env* precursor gp160. The monoclonal antibody to HIV reverse transcriptase (M3364) was used as a negative control and also did not precipitate p39. Thus, p39 contains epitopes present on both p17 and p24 (*gag*) and is unrelated to gp41 (*env*) and reverse transcriptase.

Posttranslational modifications of *gag* gene products. (i) p17 is myristylated. We have mentioned above that p17 is inaccessible to Edman degradation. To elucidate the nature of its

TABLE 1. Basic features of anti-HTLV-III$_B$ p24 and p17 hybridomas

Monoclonal antibody	Subtype[a]	Specificity	
		Antigens recognized	Method
M26	IgG1 κ	p24, p53	Immunoblot
		p24, p53	RIP
M33/1E2	IgG2a κ	p17, p53	Immunoblot
		p17, p53	RIP

[a] IgG, Immunoglobulin G.

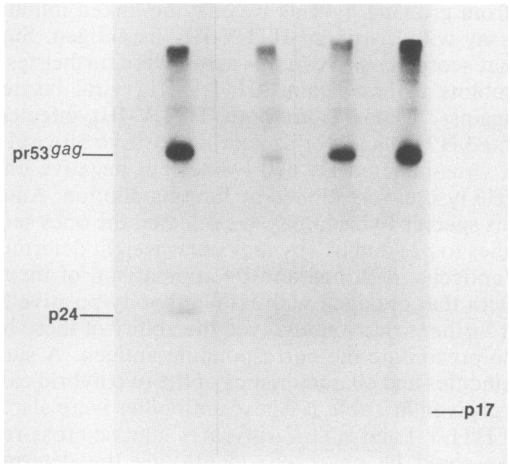

FIG. 3. RIP and SDS-PAGE analysis of HIV *gag* gene products from [^{35}S]cysteine-labeled H9/HTLV-III$_B$ cells by using monoclonal anti-p24 and anti-p17 antibodies. Tissue culture cells were radioactively labeled by overnight incubation in cysteine-free RPMI 1640 containing 100 μCi of [^{35}S]cysteine per ml. RIP was performed as described in Materials and Methods, and the samples were loaded on a 12% polyacrylamide gel.

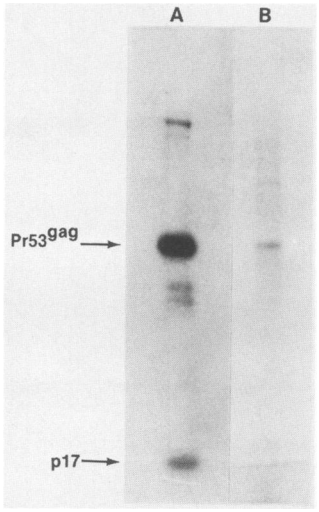

FIG. 5. RIP and SDS-PAGE analysis of [^{3}H]myristic acid-labeled H9/HTLV-III$_B$ *gag* proteins. Tissue culture cells were metabolically labeled by 2 h of incubation in RPMI 1640 containing 1 mCi of [^{3}H]myristic acid per ml. RIP was performed as described in Materials and Methods. Lanes: A, ascitic fluid from M33/1E2; B, ascitic fluid from M26.

NH_2-terminal modification, we investigated the incorporation of [^{3}H]myristic acid into p17, in view of the demonstrated myristylation of the NH_2-terminal *gag* protein of several retroviruses, including HTLV-I (18, 29). H9 cells producing HTLV-III$_B$ were metabolically labeled with [^{3}H]myristic acid, and the clarified cell lysate was immunoprecipitated with anti-p17 and anti-p24 monoclonal antibodies. Results are shown in Fig. 5. Both antibodies precipitated a labeled Pr53^{gag}. In addition, the anti-p17 antibody also precipitated labeled p17. No incorporation of radioactivity was evidenced in the p24 precipitated by the anti-p24 antibody, even after long exposure of the autoradiogram. This analysis clearly demonstrates that HIV p17 is also an N-myristylated protein.

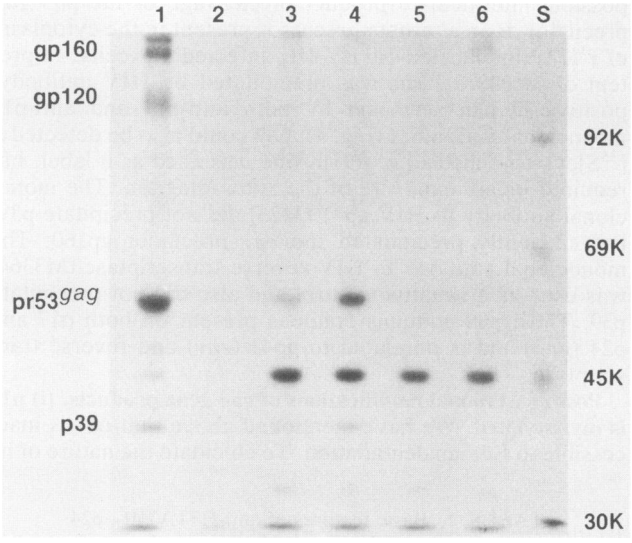

FIG. 4. RIP and SDS-PAGE analysis of HIV *gag* gene products from [^{3}H]lysine-labeled H9/HTLV-III$_B$ cells. Infected cells were metabolically labeled by overnight incubation in lysine-free medium containing 100 μl of [^{3}H]lysine per ml. RIP was performed as described in Materials and Methods, and the samples were loaded on a 7.5% polyacrylamide gel. Lanes: 1, serum from an AIDS patient; 2, negative human control serum; 3, ascitic fluid from M26; 4, ascitic fluid from M33/1E2; 5, ascitic fluid from M3364; 6, ascitic fluid from M25; S, molecular weight standards, which are (from the top) phosphorylase, bovine serum albumin, ovalbumin, and carbonic anhydrase.

(ii) p24 is phosphorylated. The prototype mammalian retroviruses (e.g., murine leukemia viruses) contain immunologically type-specific phosphoproteins of molecular weight ca. 12,000 encoded by the *gag* region. To ascertain whether this was also the case for HIV, we studied the incorporation of P_i into HIV proteins. HTLV-III$_B$–producing H9 cells were metabolically labeled with $^{32}P_i$. The lysates obtained were immunoprecipitated with monoclonal antibodies to *gag* proteins and gp41; the results are shown in Fig. 6. p24 turned out to be phosphorylated. The monoclonal antibody M26 immunoprecipitated two radioactive proteins: Pr53^{gag} and p24. The monoclonal antibody to p17 (M33/1E2) immunoprecipitated only a single radioactive band, Pr53^{gag}. No radioactive precipitate was observed when the M25 monoclonal antibody directed against gp41 was used.

DISCUSSION

The characterization of HIV p24 and p17 and their mapping to the first open reading frame of the HIV genome has been possible owing to the availability of both the complete nucleotide sequence of the HTLV-III$_B$ proviral DNA and hybridomas secreting monoclonal antibodies to the individual *gag* proteins. The sequence determined for the purified HIV p24 perfectly corresponded to the amino acid sequence predicted from the nucleotide sequence. This result, besides the obvious analogy to the known retroviral genomes and the detection of some amino acid similarities between this gene product and HTLV-I and bovine leukemia virus p24s, further identified the first open reading frame of HTLV-III$_B$ as the *gag* gene. The chemical analysis of HIV p24 showed that it had similarities with other retroviral major core proteins,

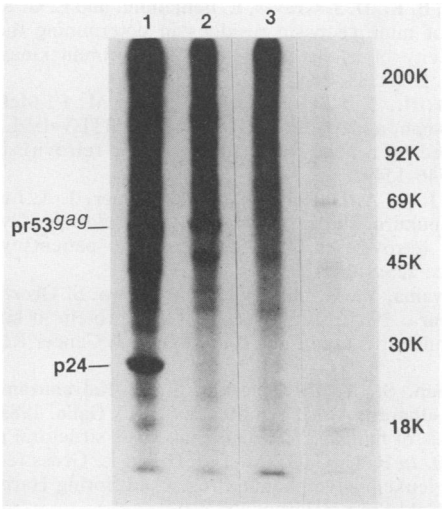

FIG. 6. RIP and SDS-PAGE analysis of [^{32}P]labeled H9/HTLV-III$_B$ proteins. Tissue culture cells were metabolically labeled by 3 h of incubation in phosphate-free RPMI 1640 containing 1 mCi of $^{32}P_i$ per ml. RIP was performed as described in Materials and Methods. Lanes: 1, ascitic fluid from M26; 2, ascitic fluid from M33/1E2; 3, ascitic fluid from M25.

but also distinct differences from them. HIV p24 shares the common proline NH_2 terminus of all retrovirus major core proteins, but has a unique NH_2-terminal amino acid sequence most closely related to equine infectious anemia virus (EIAV) and visna virus. Immunologically, a one-way cross-reactivity was recognized between HIV p24 and HTLV-II p24 and between HIV p24 and EIAV p26 (27). Both a rabbit antiserum to HTLV-II and a horse antiserum to EIAV p26 reacted with HIV p24 on immunoblots, but antibodies to HIV did not react with HTLV-II p24 or EIAV p26 under the same conditions.

Edman degradation of p17 revealed a blocked NH_2 terminus. This was consistent with the conclusion that p17 is the NH_2-terminal HIV *gag* protein. As occurs for NH_2-terminal *gag* proteins of other retroviruses, including HTLV-I and HTLV-II, HIV p17 is blocked by a posttranslational myristylation (17, 18). The structural proteins of the *gag* gene of retroviruses are derived by proteolytic cleavage of a polypeptide precursor encoded by the 5′ end of the genome, and HIV *gag* proteins are no exception. Much of the structural correlation between the precursor molecule in the infected cell and the processed proteins in the mature virion was made with the help of the two monoclonal antibodies recognizing p24 and p17, as well as Pr53^{gag}.

Use of monoclonal antibodies revealed an incomplete cleavage product or an alternate precursor of Pr53^{gag}, Pr39^{gag}, often mistaken for gp41 in immunoblots and RIP. Our data provide immunological evidence for the presence of p17 and p24 determinants in Pr39^{gag}. Findings reported by other investigators are consistent with ours and indicate that Pr39^{gag} is encoded by the NH_2 terminus of the *gag* gene (S. Venkatesan, personal communication). Another intermediate cleavage product or alternate precursor of Pr53^{gag} of approximately the same size and containing sequences from both p24 and p15, the carboxy-terminal product of the *gag* gene, has been identified in cells infected with HTLV-III$_B$ by the use of a monoclonal antibody to a fragment of p15 (35). Taken together, all these results gave insight into the processing of the *gag* gene products (Fig. 7). The primary translational product of the *gag* gene is Pr53^{gag}, which is subject to processing along either of two pathways: one by cleavage to give rise to the virion structural core proteins and the second through the intermediate Pr39^{gag} to give rise to cleavage to p17 and p24. The myristilation of p17 leads to speculation about the biological role of this protein. Myristilation in retroviral structural proteins and in two cellular proteins has been discovered (6, 29). Although myristilation of Pr65^{gag} was found to be essential for virus particle formation in Moloney murine leukemia virus (23), it was found to be required for intracytoplasmic transport of complete viral capsids to their normal site of budding and release, but not for assembly of D-type retrovirus cores (intracytoplasmic A particles) (24). The avian type C viruses and, among the lentiviruses, visna virus and EIAV, are not myristylated, arguing for other structural components to perform the same function performed by myristate. Site-directed mutagenesis experiments would help us to understand the specific function of myristate in human retroviruses.

Although myristylation of p17 was no surprise, phosphorylation of p24 was at first unexpected. In no other known retroviral system has the major core protein (the p24 homolog) been shown to be the major *gag* gene product which is phosphorylated. Analysis of the amino acid sequence of p24 reveals at least one potential phosphorylation site (Arg-X-Ser) starting with Arg-232 (14, 21). Our in vivo results are also supported by the analysis of proteins derived from HIV Pr53^{gag} for phosphoamino acids by a method involving the use of mild acid hydrolysis and anion-exchange chromatography (12). Analysis of p24 showed peaks eluting in the position of both phosphoserine and phosphothreonine, whereas all other *gag* proteins and peptides tested gave negative results. These findings suggested that p24 is phosphorylated on both serine and threonine hydroxyl groups. Viral isolates of mouse, feline, pig, woolly monkey, gibbon ape, and avian origin are characterized by immunological type-specific proteins of molecular weight ca. 12,000 (p12), encoded by the *gag* region upstream from the major core protein, which are highly phosphorylated (33). These p12s are always located after the amino-terminal *gag* protein. This is also the position of HIV p24, although it represents the major core protein.

Both p17 and p24 are immunogenic in the natural host, although p24 is more immunogenic than p17. Serum samples from most HIV infected people react with these antigens in immunological assays such as immunoblots or RIP. The latter test is extremely useful when additional supporting evidence is needed to assess a single reactivity to a single

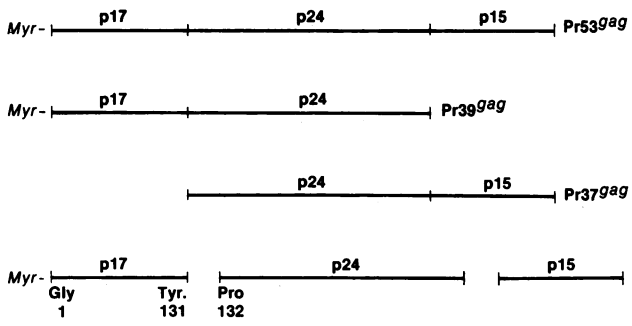

FIG. 7. Processing of *gag* gene products p17 and p24.

p24 band in immunoblots. A true reactivity to p24 in the immunoblot will be matched with a RIP profile that should show reactivity to both p24 and to its precursor in the infected cells, $Pr53^{gag}$.

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