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10. R. N. Johnston and B. L. Kucey, unpublished data.
11. Heat shock conditions used in these experiments were adapted from those described by G. C. Li and A. Laszlo [in *Changes in Eukaryotic Gene Expression in Response to Environmental Stress*, B. G. Atkinson and D. B. Walden, Eds. (Academic Press, New York, 1985), pp. 227–254]. Culture plates (100 mm in diameter, containing 10 ml of medium) with exponentially growing cells were sealed with Parafilm and placed in a 45°C circulating water bath for 20 or 30 min, as indicated; the culture medium was replaced after the heat treatment. For radioactive labeling, cells were incubated for 2 hours in the presence of 15 μCi of [^{35}S]methionine or [^{14}C]leucine per milliliter. The cells were then rinsed four times in phosphate-buffered saline, collected by scraping with a rubber policeman in 10 m*M* tris and 1 m*M* EDTA, *p*H 7.4, lysed by freezing and thawing and mixing in a vortex mixer, then centrifuged for 10 min at 12,000*g*. The trichloroacetic acid–precipitable supernatants were then analyzed. Incorporation of radioactivity in the heat-shocked samples varied from 5% to 50% of controls, with inhibition being greatest after heat shock of the CHO DH_f cell line. The lanes of heat shock proteins were therefore loaded with larger amounts of total protein to equalize radioactivity per lane.
12. The mouse monoclonal antibody used in these experiments is designated C92 [W. J. Welch and J. P. Suhan, *J. Cell Biol.* **103**, 2035 (1986)] and is reactive against both the constitutive (73-kD) and inducible (72-kD) products of the *hsp70* gene family in hamster cells. Binding of secondary antibody linked to horseradish peroxidase was detected by reaction with diaminobenzidine and with color enhancement by $CoCl_2$. Equal aliquots of soluble protein were loaded per lane.
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Unexpectedly High Levels of HIV-1 RNA and Protein Synthesis in a Cytocidal Infection

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The expression of a laboratory strain of HIV-1 (HTLV-IIIB) has been studied in mitogen-stimulated peripheral blood lymphocytes (PBLs) and in two lymphoid cell lines (CEM cells and C8166 cells). HIV-expressing cells contained from 300,000 to 2,500,000 copies of viral RNA per cell. Near-synchronous expression of an active infection could be achieved in C8166 cells. In these cells, the high copy numbers of viral RNA used as much as 40% of total protein synthesis for the production of viral *gag* protein, with high levels of viral RNA and protein synthesis preceding cell death by 2 to 4 days.

STUDIES OF HIV-1–INDUCED CELL killing have shown that HIV-1–envelope glycoproteins can kill cells by inducing the formation of syncytia (*1*). Recently we reported that HIV-1–induced cell killing does not necessarily involve syncytium formation, because mitogen-stimulated peripheral blood lymphocytes (PBLs) as well as certain lymphoid cell lines undergo HIV-1–induced cytolysis as single, mononucleated cells (*2*). The current studies were undertaken to determine whether the HIV-1 life cycle might influence the cytopathicity of this virus. The life cycles of cytopathic viruses typically result in infections using 30% or more of the protein synthesis of the host cell (*3*). This domination of host protein synthesis is achieved by viruses such as adenovirus or poliovirus actively disrupting the production or translation of host messenger RNA (mRNA) or by viruses such as vesicular stomatitis producing levels of viral RNAs (several hundred thousand copies per cell) that compete with host mRNA for translation. In contrast, typical retrovirus infections produce relatively low copy numbers of viral mRNAs (several thousand copies per cell) and use only 1 to 2% of host protein synthesis (*4*). These moderate levels of expression have been consistent with retrovirus-infected cells continuing normal growth and differentiation while producing virus.

HIV-1 can undergo latent as well as active phases of infection (*5*). In most commonly used cell cultures, simultaneously infected cells produce viral proteins and undergo cytolysis at staggered times after infection. The early stages of a typical retrovirus life cycle (adsorption and penetration, uncoating, reverse transcription, and proviral integration) require between 12 and 24 hours in growing cells (*6*). However, in growing H9 cells, CEM cells, and mitogen-stimulated PBLs, very few infected cells express viral proteins by 48 hours after infection (*2*).

Because it is difficult to study an infection in which virus expression is not synchronized, a series of cultures [H9 cells (*7*),

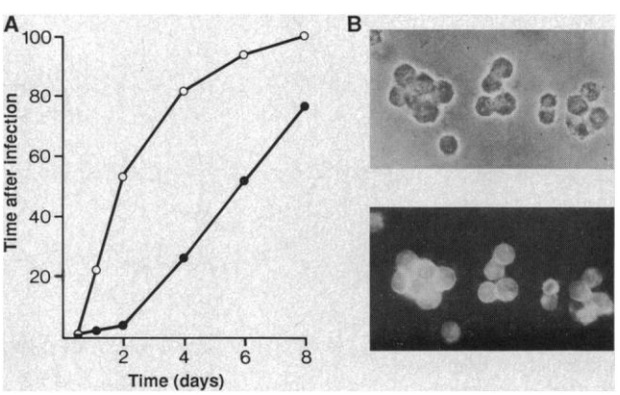

Fig. 1. Near synchronous expression of HTLV-IIIB in C8166 cells. (**A**) Time course of appearance of HTLV-IIIB protein-expressing cells (○) and dead cells (●). (**B**) Photomicrographs at 3 days after infection: phase (top) and fluorescence (bottom) microscopy. C8166 cells were infected at a multiplicity of infection of ≥0.2 infectious units of HTLV-IIIB. Leu3a (240 ng/ml) (Becton Dickinson) was added 3 hours after infection. Cultures were maintained at densities of 0.5 to 1.5 × 10^6 cells per milliliter in RPMI 1640 supplemented with 15% fetal bovine serum and 240 ng/ml of leu3a by changing the culture medium at 2-day intervals. HTLV-IIIB–expressing cells were detected by indirect immunofluorescence on fixed cells (*2*). Dead cells were defined as cells that failed to exclude trypan blue.

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CEM cells (*8*), mitogen-stimulated PBLs (*9*), Jurkat cells and *tat*-expressing Jurkat cells (*10*), T4$^+$ HeLa cells (*11*) and C8166 cells (*12*)] were tested for the ability to express the HTLV-IIIB strain of HIV-1 within 24 hours of infection. One of these, C8166 cells, had large numbers of virus-expressing cells by 24 hours after infection. Because C8166 cells are highly susceptible to HIV-1–induced syncytia, leu3a, a monoclonal antibody (MAb) that inhibits syncytium formation (*13*), was added to infected cultures. When C8166 cells were infected with ≥0.2 infectious units of HTLV-IIIB per cell and then grown in the presence of 240 ng/ml of leu3a, the majority of cells expressed viral proteins by 2 days after infection and underwent cytolysis as mononucleated cells by 6 days after infection (Fig. 1). A 3- to 4-day lag occurred between the expression of viral proteins and cytolysis.

To determine the steady-state levels of HTLV-IIIB RNA in virus-expressing C8166 cells, we prepared cytoplasmic extracts from infected cultures at 2 and 4 days after infection. Serial dilutions of the extracts as well as of a known amount of a standard RNA were hybridized on slot blots with a ^{32}P-labeled RNA probe complementary to sequences present in all spliced as well as unspliced HIV-1 RNAs (*14*) (Fig. 2). The amount of viral RNA in each extract was then estimated by comparing autoradiographic signals obtained for the extract with those for the standard RNA. These estimates were used to calculate the number of HIV-1 RNAs per productively infected cell (Table 1) (*15*). Estimates of the amount of HTLV-IIIB RNA per virus-expressing cell ranged from 400,000 to 2,500,000 copies per cell. These values indicate that HTLV-IIIB has the potential for producing viral RNAs at levels comparable to those observed for cytopathic viruses that compete for host protein synthesis (*3*).

To determine whether high copy numbers of HTLV-IIIB RNA might occur in other cultures that undergo HIV-1–induced cytolysis, we infected CEM cells and mitogen-stimulated PBLs and analyzed them for the steady-state levels of HTLV-IIIB RNA. The MAb leu3a was not added to cultures because under our conditions HTLV-IIIB–infected PBLs and CEM cells undergo cytolysis as mononucleated cells (*2*). Cytoplasmic extracts were prepared at times after infection when cultures contained peak numbers of virus-positive cells. Extracts of the CEM cells contained an estimated 300,000 copies and extracts of the PBLs an estimated 570,000 copies of viral RNA per productively infected cell (Table 1). RNA blot analyses confirmed the high levels of HIV-1 transcripts in CEM cells. Thus the expression of high copy numbers of HTLV-IIIB RNA can occur in normal T cells as well as in at least two different T cell lines.

The high copy numbers of HTLV-IIIB RNAs in productively infected C8166 cells, CEM cells, and PBLs suggested that cytopathic HIV-1 infections might have the potential to use a large portion of host protein synthesis. To test for this, we analyzed total lysates from near synchronously infected C8166 cells for newly synthesized HTLV-IIIB proteins. Cultures were grown in the presence of [^{35}S]methionine and [^{35}S]cysteine for 30 min, and ^{35}S-labeled proteins were analyzed by gel electrophoresis (Fig. 3). Uninfected, 12-hour–infected and 24-hour–infected C8166 cells did not reveal polypeptides above the background of host cell proteins. By 48 hours after infection, a polypeptide potentially representing the 55-kD precursor to the HIV-1 capsid (*16*) had appeared. By 60 hours, this band as well as a 41-kD polypeptide represented 40% of newly synthesized protein.

Evidence that the 55-kD and 41-kD polypeptides represented full-length and partially processed precursors for *gag* proteins (*16*) was obtained by analyzing immunoprecipitates. Both were immunoprecipitated by MAbs to the p24 and p17 *gag* proteins of HIV-1 (Fig. 3). Neither was detected in the absence of a MAb or by a MAb to the p24 *gag* protein of human T-lymphotropic virus type 1 (HTLV-1). Thus, the HIV-1 life cycle is like that of other lytic viruses in that it has the potential to use a large portion of

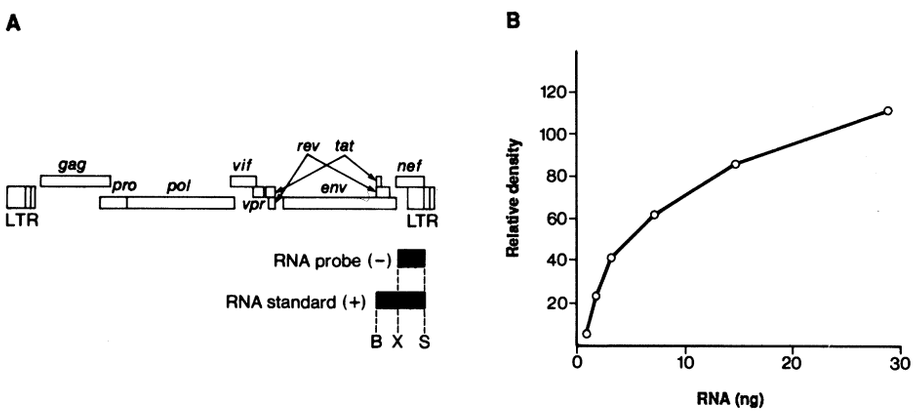


Fig. 2. Detection and quantitation of cytoplasmic HTLV-IIIB RNAs. (**A**) Regions of the HTLV-IIIB genome used to detect and quantitate RNAs (B, Bam HI; X, Xho I; S, Sac I). Nomenclature is according to Gallo *et al.* (*21*). The RNA probe contains 188 bases 5′ to the LTR, as well as the U3 region and 32 bases of the R region of the LTR. These sequences are found at the 3′ end of all HIV mRNAs (*14*). R sequences (<5% of the probe) are also found at the 5′ end of all HIV mRNAs. The RNA probe and standard were produced by using a subclone of pHXB-2 (*18*) in Bluescribe (Stratagene) and standard RNA transcription protocols (*20*). Both probe and standard RNAs were analysed on gels to verify that sizes were in agreement with the molecular weights predicted from sequence analyses. (**B**) An example of a curve used to estimate copy numbers of HIV-1 RNA. Dilutions of the RNA standard were done in the presence of uninfected C8166 cell lysates so that background hybridization would be analogous to that in cytoplasmic extracts. Densitometric tracings of autoradiographs of cytoplasmic blots were used to construct the curve.

Table 1. Steady-state levels of viral RNA in HTLV-IIIB–infected cells. Cells were infected at a multiplicity of infection of ≥0.2. HIV-1–positive cells were determined by indirect immunofluorescence with a patient serum being used as the first antibody (*2*). RNA copy numbers were determined on blots of cytoplasmic extracts prepared in the presence of 1% NP40 (*19*) and hybridized with 1×10^6 cpm/ml of the radiolabeled RNA probe depicted in Fig. 2A (*20*). Blots were digested with RNase A (1 μg/ml) in 0.3*M* sodium citrate and 0.3*M* NaCl for 15 minutes at room temperature before autoradiography. The amount of HIV-1–specific RNA in a sample was estimated by direct visualization of slot intensities relative to the intensities of the standard RNA on the same blot as well as by densitometric tracings (see Fig. 2B). The following calculation was then used: (grams of HIV-1 RNA in the sample) (Avogadro's number)/(molecular weight of the standard RNA) (number of productively infected cells in the sample) = copy number of HIV-1 RNA/productively infected cell (*15*).

Cell	Time after infection (days)	HTLV-IIIB–positive (%)	Copies of HTLV-IIIB RNA per HTLV-IIIB–positive cell
		Experiment 1	
C8166	2	70	400,000
C8166	4	100	1,300,000
		Experiment 2	
C8166	2	80	2,500,000
CEM	9	46	300,000
PBLs	6	23	570,000

host protein synthesis. This potential was not influenced by leu3a, because HTLV-IIIB–infected C8166 cells grown in the presence or absence of leu3a expressed similar levels of HTLV-IIIB *gag* protein.

To test whether the HIV-1 life cycle might have the potential to actively inhibit host protein synthesis, we examined the apparent rate of protein synthesis at various times after infection by comparing the amounts of ^{35}S-labeled amino acids incorporated into protein by infected and uninfected C8166 cells during a 15-min incubation period (Table 2) (*17*). Cultures of C8166 cells consistently showed 10 to 20% reductions in the apparent rate of protein synthesis at 24 hours, and ~50% reductions in the apparent rate of protein synthesis at 48 hours after infection. By 72 hours after infection, the apparent rate of protein synthesis had returned to normal. This "normal" rate of protein synthesis did not reflect normal levels of host protein synthesis because by this time, 40% of the newly synthesized protein was viral *gag* protein (Fig. 3). These results raise the possibility that HIV-1 has the potential to actively inhibit as well as compete for host protein synthesis. Proof of this will require demonstration of the mechanism by which HTLV-IIIB reduced the apparent rate of protein synthesis in 48-hour–infected C8166 cells.

Our results show that cytopathic HIV-1 infections produce high levels of viral RNAs that can use large portions of host protein synthesis (Table 1, Fig. 3). Our experiments also suggest that HIV-1 infections have the potential to actively interfere with host protein synthesis (Table 2). The utilization of a large portion of cell protein synthesis by a viral infection has always been correlated with the infection killing the cell. Thus, our results suggest that HIV-1–induced cell killing may in part be determined by a life cycle that allows the production of high levels of viral RNA and proteins.

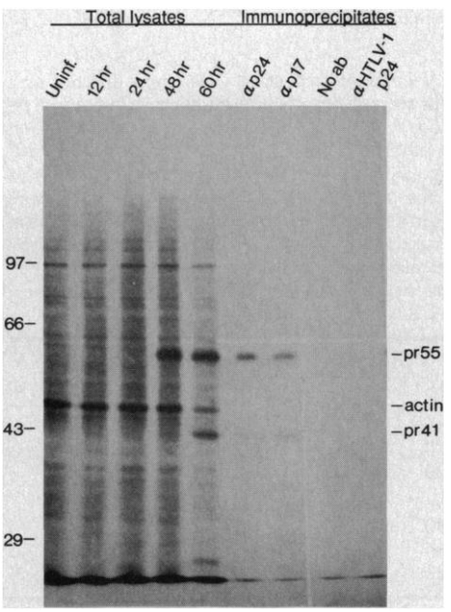
Such high levels of viral RNA and protein synthesis have not been reported in previously studied retrovirus infections. Unlike more moderate retrovirus infections, cytopathic HIV-1 infections accumulate several hundred copies of unintegrated viral DNA (*18*). We suggest that HIV-1 has the ability to actively express this DNA and that it is the efficient expression of unintegrated DNA that allows HTLV-IIIB to produce unexpectedly high levels of viral RNA and protein.

Table 2. Analysis of protein synthesis in HTLV-IIIB–infected C8166 cells. Protein synthesis was determined on duplicate samples of cells independently grown in the presence of ^{35}S-labeled amino acids for 15 min as described in Fig. 3. Labeled cells were washed in phosphate-buffered saline (PBS), adjusted to 0.1*N* NaOH and incubated at 37°C for 10 min to uncharge transfer RNA. Proteins in the lysate were precipitated with trichloroacetic acid (TCA) in the presence of bovine serum albumin (0.2 mg/ml), collected on nitrocellulose filters (0.45 μm), dried under a heat lamp, and counted in a scintillation counter with Ecolume (ICN Radiochemicals).

Time after infection (hours)	HTLV-IIIB–positive (%)	Protein synthesis	
		Inf./uninf.*	*gag*/total†
12	0	1.1, 1.0	none detected
24	18	0.9, 0.7	none detected
48	75	0.5, 0.55	0.1
72	100	0.95, 1.1	0.4

*The ratio of infected to uninfected cells was determined by indirect immunofluorescence (*2*). †The ratio of *gag* protein to host cell protein synthesis was determined by using densitometric tracings of autoradiograms such as that portrayed in Fig. 3.

Fig. 3. Autoradiograph of newly synthesized proteins in HTLV-IIIB–infected C8166 cells. Cells (0.5×10^6) were washed in methionine- and cysteine-free medium and then incubated at 37°C for 30 min in 0.5 ml of the same medium supplemented with 50 μCi of ^{35}S-Translabel (SA 1013 Ci/mM, ICN Radiochemicals). Incorporation of ^{35}S was linear with time of incubation. Washed cells were lysed in 0.25*M* tris-HCl, 2% SDS, and 5% β-mercaptoethanol, incubated at 100°C for 3 to 5 min and aliquots containing 100,000 cpm of TCA-precipitable radioactivity analysed by electrophoresis through a 10% SDS-polyacrylamide gel. For immunoprecipitations, washed cells were lysed in PBS containing 0.001*M* EDTA, 1% NP-40, and 0.5% sodium deoxycholate. Aliquots (50 μl) of the lysate were incubated at room temperature for 60 min with 1 ng of the designated MAbs (Dupont) and then for an additional 60 min with rabbit antibody to mouse immunoglobulin adsorbed to sepharose beads. The beads were washed thrice with PBS containing 0.5% NP40 and 0.4% SDS, resuspended in Laemmli sample buffer, boiled for 5 min, and the eluted proteins loaded on the gel. The fixed gel was treated with En^{3}hance (New England Nuclear), dried, and exposed to x-ray film in the presence of an intensifying screen.



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15. A sample calculation of copies of HTLV-IIIB RNA per cell follows. In experiment 1, the slot blot representing day 2 after infection had a relative density of 44 for cytoplasmic extract representing 3.3×10^4 cells and of 27 for 1.1×10^4 cells. By using the curve in Fig. 2B, these optical densities were estimated to represent 4.2 ng and 2.1 ng of HIV-1 RNA, respectively, or an average of 15.75 ng of HIV-1 RNA for 1×10^5 cells; 70% of these cells were HIV-1–expressing. The calculation for this sample was: $(15.75 \times 10^{-9}$ g) $(6.023 \times 10^{23}$ molecules)/(1097 nt × 340 g per nucleotide) $(7 \times 10^4$ virus-expressing cells) = 3.63×10^5 molecules per cell, which has been rounded to 4×10^5.
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17. Determination of actual as opposed to apparent rates of protein synthesis requires determination of the specific activity (SA) of the amino acid pool used for protein synthesis, a pool which does not necessarily reflect the SA of total intracellular amino acids [for a discussion of determination of rates of protein synthesis, see D. E. Rannels, J. B. Li, H. E. Morgan, L. S. Jefferson, in *Methods Enzymol.* **37**, 239 (1975)]. To test whether the SA of the externally added [^{35}S]methionine influenced the apparent rates of protein synthesis, we repeated the experiment in Table 2 with 50 μCi of [^{35}S]methionine diluted with unlabeled methionine to a specific activity 2×10^6 times lower than that used in the experi-

ment in Table 2. The results of this experiment confirmed the results in Table 2, revealing a 70% decrease in the apparent rate of host protein synthesis prior to the onset of high levels of HTLV-IIIB protein synthesis and a return to "normal" rates of protein synthesis accompanying the synthesis of high levels of *gag* protein.
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Human T Cell Leukemia Viruses Use a Receptor Determined by Human Chromosome 17

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Human T cell leukemia viruses (HTLV-I and HTLV-II) can infect many cell types in vitro. HTLV-I and HTLV-II use the same cell surface receptor, as shown by interference with syncytium formation and with infection by vesicular stomatitis virus (VSV) pseudotypes bearing the HTLV envelope glycoproteins. Human-mouse somatic cell hybrids were used to determine which human chromosome was required to confer susceptibility to VSV(HTLV) infection. The only human chromosome common to all susceptible cell hybrids was chromosome 17, and the receptor gene was localized to 17cen–qter. Antibodies to surface antigens known to be determined by genes on 17q did not block the HTLV receptor.

HTLV-I IS ETIOLOGICALLY ASSOCIATED with adult T cell leukemia-lymphoma (*1*) and tropical spastic paraparesis (*2*), while HTLV-II is associated with T cell hairy leukemia (*1, 3*). Although only T cells appear to be sensitive in vitro to transformation (immortalization) by HTLV (*4*), many human and mammalian cells can be infected including sarcoma cell lines, and epithelial and endothelial cells (*5, 6*). The only retroviral receptor molecule unequivocally identified to date is the CD4 leukocyte antigen, which is used by human and simian immunodeficiency viruses (*7*).

For HTLV-I and HTLV-II, cell surface receptors can be detected by assays of syncytium induction (*6*), virion binding (*8*), and VSV pseudotypes (*9*). VSV(HTLV) pseudotypes acquire the host range and receptor specificity of HTLV detected by VSV plaque formation. Cells chronically producing retroviruses express viral envelope glycoproteins that mask or down-modulate receptor expression at the cell surface. We made use of this phenomenon, known as receptor interference (*1, 7, 9*) to confirm that HTLV-I and II used a common receptor and investigated whether its determining gene or genes could be mapped.

Human and hamster cells expressed HTLV-I and II receptors, whereas mouse cells, the bovine MDBK line, and the rat NRK line were relatively resistant (Table 1). Human cells productively infected with the PL and PK isolates of HTLV-I (HOS/PL, HT1080/PK) showed receptor interference with pseudotype plating and syncytium induction of HTLV-I and HTLV-II. From these results and previous findings with cat cells (*9*), we conclude that HTLV-I and HTLV-II bind to the same receptor, whereas bovine leukemia virus (BLV) uses a distinct receptor. This accords with the antigenic relation (*9, 10*) between the gp46 outer envelope glycoprotein of HTLV-I and II, which is thought to interact with the receptor.

Since plating efficiency of VSV(HTLV) on murine cells was approximately 1% that of human cells, we used human-mouse somatic cell hybrids to assign the receptor gene or genes to a human chromosome. VSV pseudotypes have previously been used for the assignment of retrovirus receptor genes to mouse chromosomes (*11*) and to human chromosome 19 (*12*). The only human chromosome common to each of the seven sensitive hybrid cell lines was chromosome 17 (Table 2). Among the fifteen insensitive hybrid lines, however, two contained human chromosome 17. The presence of chromosome 17 in these two hybrids was confirmed by expression of the MIC6 cell surface antigen detected by flow cytometry with monoclonal antibody H207 (*13*). The receptor gene in these hybrids may either be deleted or not expressed. Two cell hybrids, PCTBA1.8 and GPT17/3, were independently derived and carried chromosome 17 as the only human genetic contribution (*13, 14*). These hybrids were sensitive to both VSV(HTLV-I) and VSV(HTLV-II). The gene determining the HTLV receptor can therefore be assigned to human chromosome 17.

The sensitivity of human-mouse somatic hybrids containing fragments of chromosome 17, as shown in Fig. 1, was tested to obtain a regional localization of the receptor gene. Since the hybrid P7A/2 (*15*) lacking the whole of the short arm of chromosome 17 other than the centromeric region, was sensitive to VSV(HTLV-I and II), the receptor gene can be localized to 17cen–qter. Furthermore, selection against chromosome 17 in this hybrid (*15*) conferred resistance to these pseudotypes. Two hybrids, PJT2A1

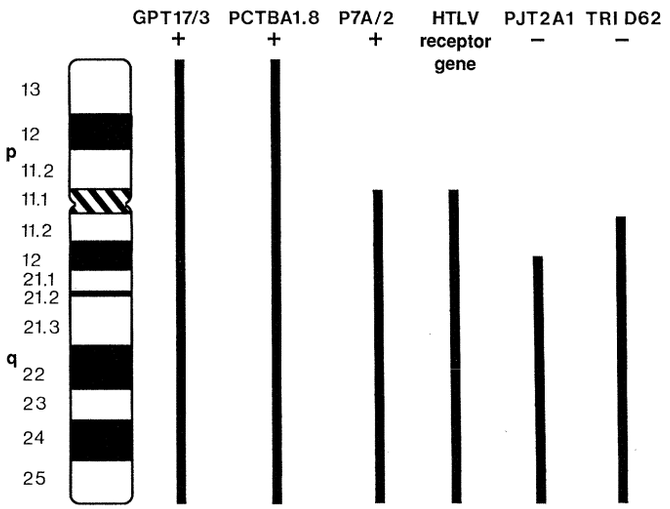

Fig. 1. Regional localization of the HTLV receptor gene on chromosome 17. VSV(HTLV-I) and VSV(HTLV-II) pseudotypes were plated on somatic cell hybrids (*14–16*) containing different portions of human chromosome 17. Susceptibility (+) and resistance (–) was scored as for Table 2.

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