$P[>w^{hs}>]$ (*15*).

It has been shown that FLP can excise a chromosomally integrated copy of the *white* gene flanked by directly repeated copies of an FRT. This resulted in somatic and germinal mosaicism for the cloned *white* gene and thus provided a simple and efficient method by which one could make mosaics of cloned genes. My results now extend those findings to show that FLP can mediate exchange between FRTs on homologous chromosomes. With the use of three marker genes (*yellow, Stubble*, and *multiple wing hairs*), I have shown that the mosaicism produced by FLP-catalyzed mitotic recombination can occur in virtually every epidermal tissue and in the germ line of *Drosophila*.

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6. $P[>w^{hs}>]2A$ and $P[>w^{hs}>]75A$ are insertions of the *P* element carrying the FRT-flanked *white* gene on the left (L) and right (R) arms of chromosome 3, respectively. The positions of these $P[>w^{hs}>]$ insertions have been determined by genetic mapping and have been inferred from the observation that they can be used to generate mosaics for genes located on chromosome 3L and 3R, respectively, as described here. $P[ry^+, hsFLP]$ is a *P* element carrying the ry^+ eye color gene and the heat-inducible *FLP* gene; $P[ry^+, hsFLP]1$ and $P[ry^+, hsFLP]2B$ are insertions of this element on the X chromosome and chromosome 2, respectively, and are referred to simply as *hsFLP*1 and *hsFLP*2B. The construction of these *P* elements has been previously described (*5*).
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11. For these experiments, females of the genotype w^{1118}/w^{1118}; *hsFLP*2B/+; *mwh* $P[>w^{hs}>]2A/mwh^+P[>w^{hs}>]2A$ were generated. Wings were mounted and examined at a magnification of ×250. For the non–heat shock experiment, pairs of wings from 24 flies were examined. For the heat shock experiment, the left wings from five flies were examined at ×500; in this case, the homozygous *mwh/mwh* cells were quite numerous, and so, rather than attempt to count separate clones, all the *mwh/mwh* cells on the dorsal surface of the wing in the region between the third and fourth longitudinal veins and distal to the anterior cross-vein were counted as representative of the entire wing. It has been estimated (*9*) that there are ~2200 cells in this region of the wing, and so to estimate the fraction of homozygous *mwh/mwh* cells, I divided the median of the five values by 2200.
12. Clones of cells exhibiting the *mwh* phenotype arise spontaneously in control mwh/mwh^+ animals that do not carry *hsFLP* or FRTs. The frequency varies somewhat from strain to strain, but is typically between 0.3 [J. Szabad, I. Soos, G. Polgar, G. Hejja, *Mut. Res.* **113**, 117 (1983)] and 0.04 clones per wing (*9*). I observed 0.25 clones per wing in w^{1118}/w^+; mwh/mwh^+ females. Twelve pairs of wings were examined.
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LAV Revisited: Origins of the Early HIV-1 Isolates from Institut Pasteur

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Two of the first human immunodeficiency virus type–1 (HIV-1) strains isolated were authenticated by reanalyzing original cultured samples stored at the Collection Nationale de Culture des Microorganismes as well as uncultured primary material. Cloned polymerase chain reaction products were used to analyze coding sequences of the V3 loop in the gp120 glycoprotein. The original isolate HIV-1 Bru, formerly called LAV, was derived from patient BRU. HIV-1 Lai was derived from patient LAI and contaminated a HIV-1 Bru culture between 20 July and 3 August 1983. The culture became, in effect, HIV-1 Lai, identifiable by a unique motif in the V3 loop. Because of this contamination two, rather than one, HIV-1 isolates were sent to the Laboratory of Tumor Cell Biology at the National Cancer Institute on 23 September 1983. Original HIV-1 Bru was indeed present in the sample marked JBB/LAV. However the M2T−/B sample harbored HIV-1 Lai, a strain capable of growing on established cell lines. The striking similarity between HIV-1 Lai (formerly LAV-Bru) and HTLV-3B sequences remains.

INEVITABLY, PERHAPS, THE ORIGINS OF the first HIV-1 isolates have attracted attention. Lymphadenopathy-associated virus (LAV) was isolated in January 1983 (*1*). With the agreement on the nomenclature of acquired immunodeficiency syndrome (AIDS) virus isolates, LAV became known as HIV-1 Bru (*2*). Twelve further HIV-1 isolates were derived by May 1984 from all the high risk groups described at that time. Among the first were HIV-1 DL (*3, 4*), HIV-1 Lai (*4, 5*), HIV-1 EL (*3, 4*), HIV-1 Eli (*4, 6*), and HIV-1 Ndk (*4, 7*). Together with serological analyses (*8*) and the realization of the CD4 lymphotropic and cytopathic nature of these viruses (*9*), these data demonstrated that LAV was the etiological agent of AIDS. The first reports of HIV-1 isolates from the United States were published in the spring of 1984. These included the human T cell lymphotropic virus type 3B, otherwise known as HTLV-3B, from the Laboratory of Tumor Cell Biology (LTCB) at the National Cancer Institute, Bethesda (*10–13*). LAV-like viruses were also demonstrated in reports from the Centers for Disease Control in Atlanta (*14*) and the University of California School of Medicine, San Francisco (*15*).

The molecular cloning of HTLV-3B (*16, 17*) and LAV (*18*) was followed by the publication of sequences (*19–21*). The striking similarity between the two was clearly a separate situation from the divergence usually seen between independent strains, highlighted by the sequence of the HIV-1 SF2 strain (*22*). Subsequent work on the genetic

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diversity of HIV-1 strains (*6, 23–26*), the identification of HIV-2 (*27*) and the inclusion of these viruses in the lentiviral subfamily of retroviruses (*28–31*), all indicated extensive genetic variation as a hallmark of these viruses.

Most HIV-1 isolates may be characterized by viral neutralization assays (*32*). Because of their similarity, LAV and HTLV-3B cannot be distinguished by these assays. A principal neutralizing determinant (PND) for antibodies has been defined for the viral gp120 sequence (*33–36*). This sequence is located in the V3 loop, a stretch of 33 to 37 amino acid residues linked by a disulfide bridge at its base (*37*). In the middle of this region is a conserved tripeptide with the amino acid sequence GPG (Fig. 1). The surrounding sequences are highly variable between strains and even within the same sample taken from an individual (*38–40*). LAV and HTLV-3B V3 loop sequences remain the only sequences out of more than 600 analyzed from the United States, Europe, and Africa to possess an insertion of two residues (indicated in boldface) before the GPG tripeptide that yield the IRI**QR**GPGRAFV motif (*38, 40*).

Our attention was drawn to a recent report (*41*) indicating that LAV samples sent to the LTCB in the summer of 1983 did not bear the hallmarks of the published LAV sequence (*19*), notably the IRIQRGPGRAFV sequence (*38*). In view of this it was decided to authenticate the original isolates from Institut Pasteur. The information pertinent to isolation and passage of the early 1983 HIV-1 strains, exchanges between Institut Pasteur and the LTCB, as well as the samples used in this study are shown in Table 1. These samples were all derived in the period before 15 May 1984, when HTLV-3B was received by Luc Montagnier. They include a DNA sample from a lymph node of patient BRU taken in January 1983, and four isolates [LAV, immunodeficiency-associated virus–number one (IDAV-1), IDAV-2, and B-LAV] deposited at the Collection Nationale de Culture des Microorganismes (CNCM). Furthermore, M2T−/B viral supernatant was also analyzed. M2T−/B was one of the two samples sent to the LTCB in September 1983 and yet was missing from their recent study. We had kept tubes of the viral supernatant corresponding to the same culture.

At the genetic level, retroviruses, like other RNA viruses, need to be defined as populations of viral genomes, referred to as quasispecies (*42*). Available data indicate that HIV isolates should be so described (*43–49*). Most of these studies have relied on sequencing small polymerase chain reaction (PCR)–amplified products. The appropriate choice of target sequences permitted the characterization of isolates (*44*). We chose the V3 loop because it includes the LAV-derived IRIQRGPGRAFV motif. PCR was performed either on extracted cellular DNA or on viral supernatants (*50*). PCR products were cloned into bacteriophage M13 and approximately 20 clones from each sample were sequenced (*51*) (Fig. 1). There were two groups of sequences. The first included sequences from the BRU lymph node and JBB/LAV derivatives (Table 1). The report from the LTCB (*41*) confirmed the presence of this sequence in JBB/LAV sent to them by Institut Pasteur, although not with perfect fidelity (*52*). This authentic HIV-1 Bru, derived from the patient known as BRU, has the V3 loop "signature" of IHIGPGRAFY.

None of the first group of sequences, which represented authentic HIV-1 Bru, resembled the distinctive LAV/HTLV-3B V3 loop sequences first published in January 1985 (*19–21*) (Fig. 1). Yet the IRIQRGPGRAFV motif was found in later passages of LAV (JBB2′/LAV, 5 August 1983;

		10 20 30	
BRU Lymph node Jan 83	A046	CTRPNNNTRKSIHI--GPGRAFYATGEIIGDIRQAHC	57%
	A051	.....N.--.....T.....	15%
	A045	.....--.....T.....R.....	5%
	A049	....S.....--.....	5%
	A053	.....--.....T.....	5%
	A055	.....--.....K.....	5%
	A060	.....P.--.....T..D.....	5%
	A064	.....--.....H...	5%
JBB/LAV Sept 83	D24	.....--.....	90%
	D29	.....D.....--.....	5%
	D30	.....R.--.....	5%
JBB/LAV Dec 90	B067	.....--.....	100%
PBMCs LAI Aug 83	L7	.....R.QR.....VT..K.-..M.....	35%
	L3	.....R.QR.....VTI.K.-.NM.....	15%
	L2	.....R.QR.....VT..K.-.NM.....	5%
	L6	.....R.QR....T.VTI.K.-.NM.....	5%
	L9	.....R.HR.....TI.K.-.NM.....	5%
	L4	.....P.--.....T.....	25%
	L20	.....--.....T.....N.....	5%
	L16	.....R.QR.....T.....	5%
IDAV-1 Aug 83	E70	.....R.QR.....VTI.K.-.NM.....	75%
	E79	.....R.QR.....VTI.K.-..M.....	15%
	E69	.....R.QR.....VTI.K.-RNM.....	5%
	E86	.....G...R.QR.....VTI.K.-.NM.....	5%
JBB2′/LAV Aug 83	M401	.....R.QR.....VTI.K.-.NM.....	92%
	M415	.....R.QR.....VTI.K.-..M.....	4%
	M416	.....R.QR.....VTV.K.-.NM.....	4%
M2T-/B Aug 83	K140	.....R.QR.....VTI.K.-.NM.....	63%
	K139	.....R.QR.....VTI.K.-.NV.....	5%
	K138	.....R.QEE.K...VTIRK.-EN.....	5%
	K145	.....C.QR.....VTI.K.-.NM.....	5%
	K147	.....R.QR.....VTI.K.-.N.....Y	5%
	K155	.....G.R.QR.....VTI.K.-.NM.....	5%
	K144	.....R.QR.....VTI.K.-..M.....	5%
	K152	.....--.....VG....	5%
JBB6/LAV Oct 83	C8	.....R.QR.....VTI.K.-.NM.....	95%
	C5	.....R.RR.....VTI.K.-.NM.....	5%
JBB8EBV/LAV Nov 83	G93	.....R.QR.....VTI.K.-.NM.....	95%
	G104	.....G...R.QR.....VTI.K.-.NM.....	5%
B-LAV May 84	H117	.....R.QR.....VTI.K.-.NM.....	81%
	H116	.....R.QR.....VTL.K.-.NM.....	5%
	H127	.....R.QR.....VTI.K.-ENM.....	5%
	H130	.....D.....R.QR.....VTI.K.-.NM.....	5%
	H135	.....-.....R.QR.....VTI.K.-.NM.....	5%
LAV	J19	.....R.QR.....VTI.K.-.NM.....	(*19*)
HTLV-3B	BH10	.....R.QR.....VTI.K.-.NM.....	(*20*)
	BH8	.....K.R.QR.....VTI.K.-.NM.....	(*20*)
	HXB2R	.....R.R.QR.....VTI.K.-.NM.....	(*66*)

Fig. 1. Identification of the IRIQRGPGRAFV amino acid motif amid ten quasispecies of V3 loop sequences. All sequences, shown in single-letter code (*65*), have been aligned with respect to the major sequence A046 present in the uncultured BRU lymph node. Only sequences encoding amino acid substitutions are shown. Dots indicate sequence identity; we have introduced gaps (dashes) to maximize sequence identity. On the left are the sequence codes. On the right, the proportion of clones in each quasi species that contain the given amino acid sequence is shown. At the bottom are the corresponding V3 loop sequences from LAV/HTLV-3B proviruses cloned in phage λ (*19–21, 66*). There are three variant sequences: (i) those identical to J19 (*19*) (derived from LAV) include BH10 and PV22 (from HTLV-3B) (*20, 21*), BRUNL43 (*40*) (from LAV), and HIVmfA (from HIV-1 MF) (*57*); (ii) those identical to BH8 (*20*) include HXB3 (*66*) and PV22cDNA (*21*) (all of which were derived from HTLV-3B); and (iii) the single HXB2R sequence derived from HTLV-3B (*20*). The complexity of the quasispecies in vivo is greater than those ex vivo (*45*). It should be recalled that the IDAV-1 (HIV-1 Lai) culture predates the LAI PBMC sample by 2 months (Table 1). Although the L16 sequence (PBMCs LAI) seems to be a recombinant between an L7- and an L4-like sequence, the possibility that it arose during PCR amplification of a heterogeneous sample cannot be excluded (*67*). Some sequences may be functionally defective, such as K145 or K147. The K138 sequence is an example of a G → A hypermutated sequence (*49*). A total of 217 V3 clones were sequenced; for the group with the A prefix there were 21 sequences; for B, 17; for C, 19; for D, 20; for E, 20; for G, 18; for H, 21; for K, 19; for L, 20; and for M, 22. Twenty clones from IDAV-2 (HIV-1 DL) were also analyzed (*69*).

JBB6/LAV, 31 October 1983; and JBB8EBV/LAV, 13 November 1983) and in the M2T−/B sample. Ninety-five percent of the M2T−/B sequences bore the IRIQRGPGRAFV motif. The remaining 5%, characterized by the sequence K152, represented a variant of an HIV-1 Bru–like sequence. Inspection of the V3 and flanking sequences showed only a 1.5% average divergence from an authentic HIV-1 Bru sequence. Unexpectedly, the V3 loop sequence derived from the third AIDS virus isolated at Institut Pasteur, HIV-1 Lai (IDAV-1, isolated from patient LAI, Table 1), was indistinguishable from this second group of sequences bearing the IRIQRGPGRAFV motif.

The authenticity of the HIV-1 Lai isolate was confirmed by sequencing amplified DNA from a frozen sample of peripheral blood mononuclear cells (PBMC) from patient LAI that had been stored at l'Hôpital La Pitié-Salpétrière, Paris, and supplied by J.-C. Gluckman and D. Klatzmann. The familiar IRIQRGPGRAFV sequence was again clear, indicating that HIV-1 Lai (formerly IDAV-1) was derived from patient LAI. However, it appears that patient LAI might have contained two strains characterized by different V3 motifs. Sixty-five percent of clones carried the IRIQRGPGRAFV motif, whereas the remainder represented variants of the V3 consensus sequence (*38, 40*). Analysis of the flanking sequences suggested the existence of a sequence distinct from authentic HIV-1 Bru (data not shown).

To confirm that M2T−/B was the source of the cluster of LAV/HTLV-3B sequences, we amplified and sequenced three other regions from M2T−/B that carried other motifs characteristic of LAV/HTLV-3B: the first and second hypervariable regions of the *env* gene, the first coding exon of the *tat* gene, and the region encompassing the *gag-pol* overlap (Fig. 2). The sequence identity to LAV (HIV-1 Lai) was striking. The first *env* hypervariable region encoded a sequence characteristic of LAV/HTLV-3B, including the telltale NTNSSNTNSS direct repeat (*19*). Finding envelope sequences similar to HIV-1 Lai and HIV-1 Bru supports the idea that the M2T−/B sample contained both HIV-1 Bru and HIV-1 Lai strains. The *tat* sequences were also characteristic of LAV (*19*). Examination of the *tat* PCR products that, after end-labeling with ^{32}P and digestion with Hind III or Sac I, revealed the restriction site polymorphisms (*23*) first observed in 1985 (data not shown). For the *gag-pol* overlap, two PCR bands were found at an approximately equal ratio. This was due to a 36-bp repeat unique to LAV/HTLV-3B. Thus the group of sequences that have been referred to as LAV/HTLV-3B are derived from M2T−/B and therefore from HIV-1 Lai.

That M2T−/B contained HIV-1 Lai and HIV-1 Bru sequences is the clearest evidence of contamination. A few months after the contamination is thought to have occurred, different samples form the same laboratory contained bona fide HIV-1 Bru (JBB/LAV, 27 September 1983) or the contaminating HIV-1 Lai strain (JBB6/LAV or JBB8EBV/LAV). However, both were considered as authentic HIV-1 Bru (LAV), as their designations suggest.

Table 1. Abbreviated chronology of the first three HIV-1 isolates at the Institut Pasteur. The dates shown in italics correspond to samples analyzed in this study. JBB identifies a donor whose PBMCs were extensively used in 1983. JBB2 refers to the second aliquot of these PBMCs, JBB3 to the third, and so forth. EBV, Epstein-Barr virus.

Date	Event
3 January 83	Lymph node biopsy from patient BRU put into culture. BRU was a homosexual with lymphadenopathy. He was last in the United States in 1979. First positive RT activity on 15 January. Isolate became LAV and, in turn, HIV-1 Bru. At one time referred to as RUB.
25 May 83	PBMCs from DL (a hemophilic B patient with AIDS) put into culture. DL culture RT-positive from 30 May. Isolate referred to as IDAV-2 and, in turn, HIV-1 DL.
9 June 83	Lymph node biopsy from patient LAI put into culture. LAI was a homosexual with AIDS and Kaposi's sarcoma. He had visited the United States several times between 1977 to 1979. LAI culture RT-positive from 14 June. Isolate referred to as IDAV-1 and, in turn, HIV-1 Lai.
26 June 83	LAV (HIV-1 Bru) infection of JBB donor cells. Virus passage referred to as JBB/LAV.
7 July 83	DNA extracted from part of the 26 June culture. Sent to LTCB in July 83.
12 July 83	Supernatant taken from 26 June culture. Sent to the LTCB in July 83 and September 83.
15 July 83	Supernatant taken from 26 June culture. Deposited with the CNCM, reference I-232, the same day. Sample given to Centers for Disease Control in Atlanta on 27 July.
20 July 83	Infection of JBB2′ (donor PBMC, second aliquot) by HIV-1 Bru (LAV-1). RT-positive on 29 July and 8 August. Infection of JBB2′ by HIV-1 Lai (IDAV-1) RT-positive from 25 July to 5 August. Infection of JBB2′ by HIV-1 DL (IDAV-2) RT-positive from 25 July to 12 August.
1 August 83	Uncultured PBMC sample from patient LAI stored.
12 August 83	Infection of JBB4 by IDAV-1. RT activity at 280,000 cpm per milliliter on 19 August. Supernatant stored for CNCM. Infection of JBB4 by IDAV-2. RT activity at 163,000 cpm per milliliter on 19 August. Supernatant stored for CNCM. Both supernatants deposited with the CNCM as IDAV-1 (reference I-240) and IDAV-2 (reference I-241) on 15 September 1983.
16 August 83	Bone marrow sample M2T-infected with JBB2′/LAV with supernatant taken on 3 August 83 from culture started 20 July 83. Culture subsequently referred to as M2T−/B.
26 August 83	Supernatant from M2T−/B harvested. Sample sent in September 83 to LTCB.
23 September 83	Letter signed by M. Popovic acknowledges receipt of JBB/LAV and M2T−/B.
27 September 83	Further culture of JBB/LAV.
31 October 83	DNA from frozen cell pellet of JBB6/LAV. LAV traceable to M2T−/B.
13 November 83	LAV (traceable to M2T−/B) infection of EBV-transformed JBB8 donor cells. Culture started on 17 October gave rise to B cell–adapted LAV, referred to as B-LAV. JBB8 donor cells referred to as FR8 cells in (*63*).
29 February 84	LAV (traceable to M2T−/B) sent to R. Weiss, Chester Beatty Laboratories, London.
7 April 84	LAV given to M. Gardner, University of California at Davis.
13 April 84	LAV received by M. Martin, National Institute of Allergy and Infectious Diseases in Bethesda, Maryland. C6Tx/LAV virus stock could be traced back to M2T−/B stock. We went on to clone and sequence 4 kb of this virus (*64*).
3 May 84	Three cDNA clones (pLAV13, 75, and 82) derived from B-LAV by M. Alizon. Clones pLAV75 and pLAV82 sequenced (*64*).
9 May 84	B-LAV deposited at the CNCM (reference I-299).
15 May 84	HTLV-3B received by L. Montagnier.
6 December 90	Original HIV-1 Bru (JBB/LAV dated 15 July 83) retrieved from the CNCM and cultured on purified $CD4^+$ cells.

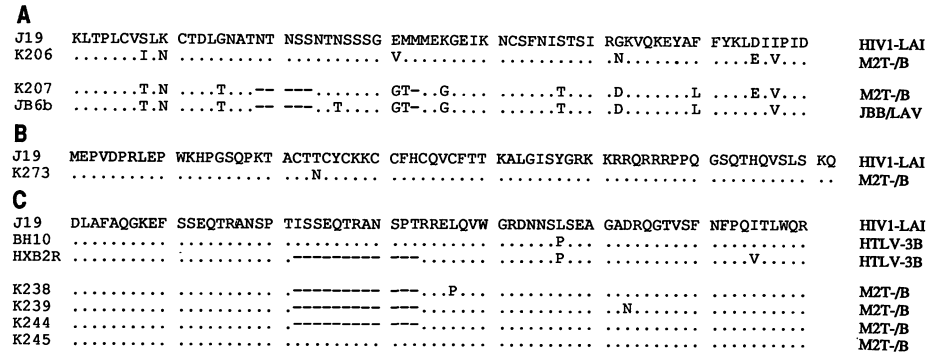

Fig. 2. Amino acid sequences of the three other regions of the proviruses derived from M2T−/B. All sequences are aligned with respect to the J19 (LAV or HIV-Lai) sequence published in January 1985 (*19*). Only amino acid differences are shown. Dots indicate sequence identity; we have introduced gaps (shown as dashes) to maximize sequence identity. (**A**) First hypervariable region in the gp120 envelope protein. JB6B was derived from JBB/LAV (authentic HIV-1 Bru) as recently reported (*41*). (**B**) The *tat* protein sequence. (**C**) NH_2-terminal Pol sequences from the region of the *gag-pol* overlap. Sequence identifiers are shown at the left; viral isolate is at the right.

Barré-Sinoussi's notebook shows that the JBB2′/LAV supernatant, believed to be authentic HIV-1 Bru (LAV), was used to infect the bone marrow culture that generated M2T−/B. This supernatant was derived from an infection started on 20 July 1983 (Table 1). However, on that same day, JBB2′ donor cells were also inoculated in parallel with HIV-1 Lai (IDAV-1) and HIV-1 DL (IDAV-2). All three were grown concurrently in roller bottles for the mass production of virus destined for enzyme-linked immunosorbent assays. The JBB2′/LAV sequences shown in Fig. 1 are characteristic of HIV-1 Lai and not HIV-1 Bru. It therefore appears that the JBB2′/LAV stock used to infect M2T−/B was contaminated by HIV-1 Lai. Recently, it was found that the JBB2′/LAV and M2T−/B virus stocks grew extremely well on the established cell line CEM, a property typical of HIV-1 Lai, rather than authentic HIV-1 Bru (data not shown). Given that M2T−/B–derived virus grew better at that time than any other stock of LAV, it is understandable that it was used for subsequent experiments.

Contamination is a recurrent problem in microbiology. There is no reason to suppose that experiments with immunodeficiency viruses are any more susceptible to contamination than experiments with other viruses in culture. Nonetheless, literature on the matter has already developed (*53–61*). From this point on, the term HIV-1 Bru will indicate the prototype HIV-1 isolated from patient BRU. HIV-1 Lai, derived from patient LAI, will be used to describe the isolate characterized by the singular IRIQRGPGRAFV motif in the V3 loop. We will change the names of our reagents accordingly. We regret this occurrence.

Patient LAI was a French homosexual who had AIDS with Kaposi's sarcoma. At the time of sampling he had a low $CD4^+$ T cell count (*5*). The virus was isolated from a lymph node biopsy. Reverse transcriptase (RT) activity was noticeable from day 5 of culture. RT titers were greater than anything that had been seen up to that time. In today's terms, such a virus would be described as a rapid-high isolate (*62*). One of the features of the HIV-1 isolates from AIDS patients is the isolate's ability to grow on established cell lines (*62*). The B cell–adapted form of LAV as described in 1984 [B-LAV derived from the JBB8EBV/LAV culture (*63*)] was in fact HIV-1 Lai. In 1985, our laboratory found that HIV-1 Lai grew well on the H9 cell line, a clone of the original HUT78 T cell line, given to us by R. C. Gallo. These growth properties may help explain how HIV-1 Lai could have successfully competed with HIV-1 Bru in culture.

The study of Guo and co-workers notes that the virus derived from the July and September samples of JBB/LAV sent by Institut Pasteur to the LTCB grew only on PBMCs (*41*). Furthermore, M2T−/B was "used in attempts at transmission by M.P. [M. Popovic] between late October and January 1984" (*41*, p. 245). The outcome of these experiments at the LTCB was not mentioned. Yet by mid-December 1983, LAV was being propagated in the HUT78 and TI7.4 established T cell lines at the LTCB. Given the properties of the M2T−/B virus (HIV-1 Lai) and the inability of LTCB to culture bona fide JBB/LAV (HIV-1 Bru) in established T cell lines, it is probable that M2T−/B was the source of LAV used to infect the HUT78 and TI7.4 cell lines. Therefore, it was surprising to note in the study of Guo and co-workers "that nothing remains of the M2T−/B supernatant, and no derivatives of it have so far been located" (*41*, p. 246).

In conclusion, two of the first HIV-1 strains ever isolated, HIV-1 Bru and HIV-1 Lai, have been authenticated. It is HIV-1 Lai from Institut Pasteur that is so widely known. Both strains were sent to the LTCB in September 1983. Might not the fate of the M2T−/B sample have some bearing on the relationship between LAV and HTLV-3B?

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50. There was sufficient DNA in a clarified viral supernatant to allow PCR without recourse to an initial

RT step. This simplified the work and reduced the risk of contamination. Presumably enough DNA, perhaps unintegrated proviral DNA, was liberated from a few dying or dead cells in the culture at the time of virus production. We heated a fraction of stored supernatant at 60°C for 30 min to inactivate the virus. Viral supernatant (5 μl) was added to a PCR reaction. The V3 loop primers BRUV5 and BRUV3 amplified all samples well. A titration of a viral supernatant with the V3 primers showed that 2 to 10 μl gave efficient amplification. However, the SK122/123 *env* primers or the GP5/3 *gag-pol* primers worked less efficiently and sometimes hardly at all.

51. The primers and probes used in this study were the following: V3 loop primers were BRUV5 (5′-GAGGAATTCAGTCTAGCAGAAGAAGAGGT-3′) and BRUV3 (5′-GGCAAGCTTGTGCGTTA-CAATTTCTGGGT-3′). The hybridization probe was BRUVP (5′-GGACCAGGGAGAGCATT-TGTTACAATAGGA-3′). A hybridization probe (SPBRU) specific for the QRGPG motif was used as a guide before sequence data was available. Its sequence was 5′-GTATCCAGAG(A/G)GGAC-CAG-3′ where (A/G) denotes a 50:50 mixture of A and G. The *gag-pol* primers were GP5 (5′-GCAG-GAATTCTTTAGGGAAGATCTGGCCTT-3′) and GP3 (5′-GACGAAGCTTGGGTCGTTGCCAA-AGAGTGAT-3′). The hybridization probe was GPP (5′-TTTCTTCAGAGCAGACCAGAGCC-AACAGCCC-3′). The V1/2 *env*-specific primers SK122 and SK123 and probe SK129, as well as the *tat*-specific primers (T1 and T2) and probes (S1, S2, and S3) have been described (*44, 45*). To eliminate the possibility of PCR contamination, DNA was extracted by colleagues working in laboratories in which an HIV plasmid had never been used. PCR reactions were carried out in a special plexiglass hood in our PCR room which housed in a separate building. Only the workers' forearms enter the hood. No HIV plasmid had ever before been manipulated in this room, nor had any tube containing amplified material ever been opened there. Tubes were opened and analyzed in the main molecular biology laboratory. Negative controls were run each time. All were negative. Reactions contained 2.5 mM $MgCl_2$, 50 mM KCl, 10 mM tris-HCl (pH 8.3), 50 μM of each deoxynucleotide triphosphate, 100 pmol of each primer, and 5 U of *Taq* polymerase (Perkin-Elmer Cetus). Reaction volumes were 100 μl, including 1 to 2 μg of DNA or 5 μl of viral supernatant; 50 μl of mineral oil was laid on top. Thermal cycling parameters were as follows: 80°C, 5 min; 45 cycles of (95°C, 30 s; 55°C, 30 s; 72°C, 30 s); 72°C 10 min. The PCR products were purified from 5% acrylamide gels, treated with kinase and ligated into Sma I–cleaved and dephosphorylated M13mp18 replicative form DNA. The ligated products were transformed into *Escherichia coli* TG1. Plaques were screened in situ with ^{32}P-labeled oligonucleotide probes. Approximately 20 positive clones from each sample were grown and sequenced by the dideoxy method. A total of 217 V3 clones were sequenced. All sequences were read twice and double-checked from the autoradiographs. Two cDNA clones, pLAV75 and pLAV82, isolated in April 1984 from the B-LAV isolate have been described (*18*). Their Pst I inserts were subcloned into the Pst I site of M13mp18 RF DNA. Recombinant clones were sequenced as described above. All the unique nucleotide sequences shown in Figs. 1 and 2 have been deposited with GenBank under accession numbers M64178 to M64223 and M64406 to M64417.
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64. Reference to the three cDNA clones derived from B-LAV (HIV-1 Lai) can be found in M. Alizon's notebook dated 3 May 1984. We have sequenced two of these cDNA clones, pLAV75 and pLAV82. They differ from the published sequence of LAV (*19*) by 1.5% (10/649 bases) and 2.2% (7/312 bases), respectively. In addition, the laboratory of M. Martin (National Institute of Allergy and Infectious Diseases, Bethesda, MD) molecularly cloned LAV from a sample denoted C6Tx/LAV. This virus passage from the Institut Pasteur can be traced back to the M2T−/B sample. M. Martin *et al.* sequenced ~4 kb from the 3′ end of the genome. Their sequence and the French LAV J19 sequence were 98% identical (*40*).
65. The single letter amino acid code is as follows: A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; and Y, tyrosine.
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68. The *Taq* polymerase error rate may be estimated to be <0.8 bases per 20 V3 loop sequences or <0.6 amino acid substitutions per 20 sequences (*44, 45*). There may be <6 minor forms, presumably, due to *Taq* polymerase error. Care must be exercised in interpreting in biological terms the sequences of minor forms. The homogeneity of the JBB/LAV July 1983 group of sequences is a good indication of the low level of *Taq* polymerase error.
69. As HIV-1 DL (IDAV-2) was cultured at the same time as HIV-1 Bru and HIV-1 Lai from 20 July 1983 onwards, the V3 loop region was amplified from a viral supernatant sample that had been deposited in the CNCM on 15 September 1983. Twenty recombinant M13 clones were sequenced. The V3 loop sequence was CTRPNNNTRERL-SIGPGRPFYATRRIIGDIRQAHC for 95% of the clones. Among 20 protein sequences a variant existed encoding a single R → K amino acid substitution at position 26. This sequence is different from those of authentic HIV-1 Bru and HIV-1 Lai and does not resemble any of the V3 sequences published to date.
70. Samples of HIV-1 Bru, HIV-1 Lai, and B-LAV have been deposited with the CNCM. Samples of each still remain. Additional vials of the M2T−/B sample are available. All samples used for amplification in this study, as well as the recombinant M13 clones, are available for corroboration if necessary.
71. We thank the following colleagues for extracting lysed DNA in their HIV-free laboratories or for performing oligonucleotide synthesis (often with little or no advance warning): M. Buckingham, S. Cole, C. Gouyette, J. Igolen, G. Langsley, B. Robert, and I. Tardieux. We thank M. Alizon for the cDNA clones and G. Myers and J. Goudsmit who educated us in the fine arts of V3 loop sequences. M. Chahine kept the laboratory running while the rest were working. Supported by grants from Institut Pasteur and l'Agence Nationale de Recherches sur le SIDA.

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Similarity of Human Mitochondrial Transcription Factor 1 to High Mobility Group Proteins

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Human mitochondrial transcription factor 1 (mtTF1) has been sequenced and is a nucleus-encoded DNA binding protein of 204 amino acids (24,400 daltons). Expression of human mtTF1 in bacteria yields a protein with correct physical properties and the ability to activate mitochondrial DNA promoters. Analysis of the protein's sequence reveals no similarities to any other DNA binding proteins except for the existence of two domains that are characteristic of high mobility group (HMG) proteins. Human mtTF1 is most closely related to a DNA binding HMG-box region in hUBF, a human protein known to be important for transcription by RNA polymerase I.

TRANSCRIPTION OF HUMAN MITOchondrial DNA (mtDNA) proceeds in opposite directions from a promoter on each strand. These promoters, the light-strand promoter (LSP) and heavy-strand promoter (HSP), are both localized to the major regulatory region for mtDNA transcription and replication (*1*). The use of an in vitro transcriptional assay of human mitochondrial extracts (*2*) coupled with deletional (*3*) and linker substitution (*4*) analyses has revealed the bipartite nature of each promoter; one domain of ~15 bp encompasses the transcriptional start site, and the other is an upstream domain of ~30 bp that is bound by the transcriptional activator protein mtTF1 (*5, 6*). This promoter structure is conserved in both human and murine mitochondria despite the relatively unconserved (<50% identity) nucleotide sequence of the mtTF1 binding sites in the two species (*6*).

Biochemical fractionation of mitochondrial transcription extracts has revealed an absolute requirement for a minimum of two proteins: a mitochondrial RNA (mtRNA) polymerase and mtTF1, which has been isolated from both human and mouse mitochondria (*6–8*). The species specificity of mitochondrial transcription appears to reside in the polymerase-containing fraction; human mtTF1 can substitute for its murine counterpart on the heterologous promoter, but only if mouse extracts containing polymerase activity are provided. Purification of

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