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11. The experimental monkeys were given two intramuscular injections of antigen (200 μg in alhydrogel) at an interval of 4 weeks; control monkeys received adjuvant alone. Two weeks later all the monkeys were challenged with 1200 cercariae. After a further 8 weeks the hepatic portal system was perfused to recover adult schistosomes. From the three vaccinated monkeys 471, 557, and 380 schistosomes were recovered (mean, 469.3 ± 51.1); from the two control animals 830 and 783 schistosomes were recovered (mean, 801.5 ± 18.5) ($P < 0.01$; *t*-test).
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14. Immunoglobulin M and subclasses of IgG were estimated in sera of mice taken just before challenge by radial immunodiffusion in gel (Meloy Laboratories, Springfield, Va.). Specific levels of IgE antibodies against the 155K antigen were measured in a solid-phase radioimmunoassay (*5*) with rabbit antibody to mouse IgE (Sera-Lab) affinity-purified against mouse IgE and labeled with ^{125}I.
15. Schistosome perfusion from the portal system was performed as described by S. R. Smithers and R. J. Terry [*Parasitology* **55**, 695 (1965)].
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Characterization of Long Terminal Repeat Sequences of HTLV-III

Abstract. *The nucleotide sequence of the long terminal repeat sequence (LTR) of the human T-cell leukemia (lymphotropic) virus type III (HTLV-III) was determined. This virus is associated etiologically with the acquired immune deficiency syndrome. The LTR was found to be 634 base pairs in length with U3, R, and U5 regions of 453, 98, and 83 bp, respectively. The proviral DNA is flanked by a 7-base-pair direct repeat. The promoter and polyadenylation signals are situated 27 and 24 base pairs upstream from the respective transcriptional initiation and polyadenylation sites. The primer binding site is complementary to transfer RNA-lysine. The LTR of HTLV-III, like that of HTLV-I, showed a limited homology to enhancer-like sequences within two genes expressed specifically in T lymphocytes, T-cell growth factor, and γ-interferon. Structural comparisons revealed that the LTR of HTLV-III is distantly related to those of HTLV-I, HTLV-II, and bovine leukemia virus.*

The human retrovirus termed HTLV-III has been etiologically linked to the acquired immune deficiency syndrome (AIDS) (*1–3*), a disease characterized by opportunistic infections and malignancies such as Kaposi's sarcoma. This syndrome is also characterized by a preferential loss of $OKT4^+$ lymphocytes (*4*), the principal cell target for HTLV-III replication (*2*). In a recent study (*3*), serum samples from 95 percent of patients with AIDS or AIDS-associated disorders reacted with HTLV-III viral proteins, whereas samples from 30 percent of normal homosexuals and less than 1 percent of normal heterosexuals showed such a reaction (*3*). Like the human T-cell leukemia (lymphotropic) virus types I and II, HTLV-III is lymphotropic; shows a particular capacity to infect T4 lymphocytes; contains a relatively large DNA polymerase (about 90,000 daltons), which is Mg^{2+} dependent; contains a major core protein of 24,000 daltons; induces formation of giant multinucleated cells; and, like HTLV-I and HTLV-II, contains a novel gene at the 3′ end of the viral genome referred to as the long open reading frame (LOR) (*5*). Among animal retroviruses, bovine leukemia virus (BLV) also shares many of these characteristics as well as actual protein sequence homologies with HTLV-I (*6*). HTLV-III also shows substantial genomic homology with visna virus (*7*), a retrovirus that causes a chronic neurological disease of sheep.

The availability of recombinant DNA clones of HTLV-III prepared in our laboratory (*8, 9*) has enabled us to study the molecular details of this virus genome. The LTR region of HTLV-III was first analyzed since this region in other retroviruses is known to include regulatory sequences for viral transcription, host cell tropism, and determination of pathogenetic capabilities (*10*). Furthermore, comparison of the HTLV-III LTR with the LTR's of HTLV-I, HTLV-II, BLV, and other retroviruses, especially members of the subfamily Lentiviridae, may provide evidence of the evolutionary origin of this virus.

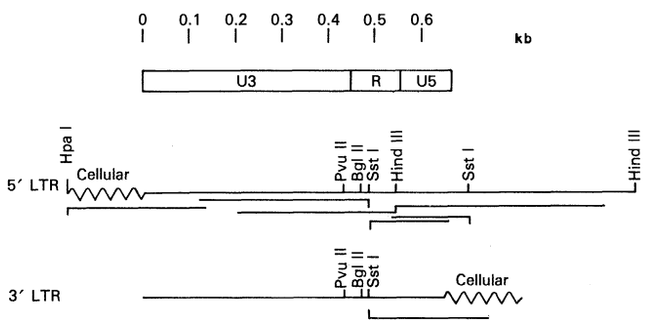

Fig. 1 (left). A schematic drawing of HTLV-III LTR, restriction map of the 5′ and 3′ LTR regions of the HXB2 (*9*) clone and sequencing strategy. To determine the cell-LTR boundaries we compared the sequencing data from an unintegrated DNA clone BH8 (*8*), corresponding to the 3′ 3.5-kb portion of the viral genome, with the sequence of HXB2. After restriction enzyme digestions, the labeling of 5′ ends was accomplished with γ-^{32}P-labeled adenosine triphosphate and the T4 polynucleotide kinase; 3′ ends were labeled with α-^{32}P dideoxy-ATP and terminal deoxynucleotidal transferase. The sequence was determined by the method of Maxam and Gilbert (*22*). The U3-R boundary was localized by S1 nuclease mapping (*23*) (see Fig. 2) and strong-stop cDNA synthesis. The R-U5 boundary was determined from the sequence of a cDNA clone obtained by using oligo(dT) as primer and viral RNA as template (not shown).

```
                                                                  TAGTAGT
U3
TGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACA    60
--
CACAAGGCTACTTCCCTGATTAGCAGAACTACACACCAGGGCCAGGGGTCAGATATCCAC   120

TGACCTTTGGATGGTGCTACAAGCTAGTACCAGTTGAGCCAGATAAGGTAGAAGAGGCCA  180

ATAAAGGAGAGAACACCAGCTTGTTACACCCTGTGAGCCTGCATGGGATGGATGACCCGG  240

AGAGAGAAGTGTTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTCATCACGTGGCCCGAG  300

AGCTGCATCCGGAGTACTTCAAGAACTGCTGATATCGAGCTTGCTACAAGGGACTTTCCG  360

CTGGGGACTTTCCAGGGAGGCGTGGCCTGGGCGGGACTGGGGAGTGGCGAGCCCTCAGAT  420

      TATA  Pvu II          U3 ▼ R                    Bgl  II
CCTGCATATAAGCAGCTGCTTTTTGCCTGTACTGGGTCTCTCTGGTTAGACCAGATCTGA  480
      ------------
                                                      Hind  III
       SstI                                        Poly(A) s
GCCTGGGAGCTCTCTGGCTAGCTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCT  540
                                                    ----------
      R  ▼ U5
TGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTC  600

                                  U5 ▼     tRNA Lys
AGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCAGTGGCGCCCGAACAGGGAC         660
                                  -- ------------------
                                     TAGTAGT
```

Fig. 2 (right). Complete HTLV-III LTR sequence of the genomic clone obtained from the HTLV-III–producing T-cell line H9 (*2*).

Figure 1a shows the structural features of the HTLV-III LTR derived from a genomic clone designated HXB2 (*9*). The complete nucleotide sequence of this LTR is shown in Fig. 1b. The complete provirus is flanked by a 7-bp direct repeat TAGTAGT. The LTR begins with the dinucleotide TG at position 1 and terminates with the inverted sequence CA at position 634. The same unusually short inverted repeats are also found in HTLV-I (*11*) and HTLV-II (*12*) LTR's and not in any of 12 other retroviruses examined including avian and mammalian type C retroviruses, the type B mouse mammary tumor virus and type D retroviruses (Table 1). The overall length of the HTLV-III LTR is 634 bp.

The U3 region is 453 bp in length, terminating at the RNA transcription initiation site as determined by S_1 nuclease mapping of the viral RNA (Fig. 2) and by estimation of the size of the (−) strand strong-stop complementary DNA (cDNA) (*13*). Upstream from this site is the proposed promoter signal, TATAA, which at −27 bp is at a position typical for promoters of eukaryotic genes (*14*). No open reading frame is found within U3. Furthermore, we were not able to find repetitive sequences in U3 such as were found in HTLV-I (*11*), HTLV-II (*12*), and BLV (*15*). Sequences related to the enhancer core sequence GTGG(A/T)(A/T)(A/T)G (*16*) include TGGTTAG at position 463 and TGGATGG at position 128.

As in the HTLV-I and HTLV-II LTR's, and again unlike that of other known retroviruses, no CAT box was found in the usual location at −70 to −80 bp from the site of RNA initiation. However, a sequence similar to that signal, CCAAT, is located upstream at position 178.

The polyadenylation signal AATAAA is positioned at −24 bp from the polyadenylation site at nucleotide 551. The placement of this signal is typical for those of eukaryotic genes (*14*) but distinct from those of HTLV-I, HTLV-II, and BLV which are positioned much further upstream (*11, 12, 15*). The R–U5 boundary was determined from the sequence of a cDNA clone obtained by using oligo(dT) as primer and viral RNA as template (not shown). The polyadenylation site is also flanked by sequences related to the transcriptional termination consensus signals TTTGCN(G/C)TTGCA and TTGT (*17*); the latter is found 20 bp downstream from this site at position 571. The R region is therefore 98 bp long.

The U5 region is 83 bp long and ends with a CA dinucleotide. This is followed by the primer site which is 18 bp complementary to the 3′ terminus of transfer RNA–lysine ($tRNA^{lys}$). This is distinct from HTLV-I, HTLV-II, BLV, and all other mammalian type C retroviruses, which utilize tRNA-proline. Mouse mammary tumor virus (MMTV) is the only other retrovirus known to contain a tRNA-lysine primer binding site (Table 1). The structural features of the HTLV-III LTR are compared to those of other retrovirus LTR's in Table 1. The lengths of U5, R, and U3 of HTLV-III are similar to those of other retroviruses thus far described.

As with the HTLV-I LTR (*18*), the HTLV-III LTR also contains sequences that have some homology to those of human T-cell growth factor (TCGF, or IL-2) (*19*). One of these sequences, which is located 275 nucleotides upstream from the HTLV-III transcriptional initiation site (CAP site, U3-R boundary), is 70 percent homologous to a sequence at an analogous position 293 nucleotides upstream from the CAP site of the TCGF gene (Fig. 4a). Other homologous (80 percent) sequences are located 20 and 24 bp downstream from the core enhancer consensus signals in the first intron of the TCGF gene and in HTLV-III U3, respectively (Fig. 4b).

A sequence similarity of 83 percent was also found between HTLV-III U3 and intron I of human γ-interferon (γ-IF) (*20*) (Fig. 4c). The corresponding sequence of the γ-IF gene is also located

Table 1. Comparison of the LTR of HTLV-III to those of other retroviruses. Abbreviations: LTR, long terminal repeat; IR, inverted repeat; HTLV, human T-cell leukemia/lymphoma virus; BLV, bovine leukemia virus; AMV, avian myelocytomatosis virus; ASV, avian sarcoma virus; RSV, Rous sarcoma virus; MoMuLV, Moloney murine leukemia virus; MSV, murine sarcoma virus; FeLV-B, feline leukemia virus B; FeSV, feline sarcoma virus; SNV, spleen necrosis virus; MMTV, mouse mammary tumor virus; SSV, simian sarcoma virus.

Virus	Length in nucleotides					tRNA primer	References
	LTR	IR	U3	R	U5		
HTLV-III	634	2	453	98	83	Lys	
HTLV-I	754	2	353	221	180	Pro	(*11*)
HTLV-II	763	2	314	247	203	Pro	(*12*)
BLV	535	6	215	233	86	Pro	(*15*)
AMV	312	9	217	8	87	Trp	(*24*)
ASV	330	15	229	21	80	Trp	(*25*)
RSV	334	15	233	22	79	Trp	(*26*)
AKR	626	13	479	70	76	Pro	(*27*)
MoMuLV	594	13	449	68	77	Pro	(*28*)
MSV	588	11	444	68	76	Pro	(*29*)
FeLV-B	539	11	394	68	77	Pro	(*30*)
FeSV	482	12	340	68	74	Pro	(*31*)
SNV	570	3	396	79	95	Pro	(*32*)
MMTV	1328	6	1194	11	118	Lys	(*33*)
SSV	504	7	360	70	76	Pro	(*34*)

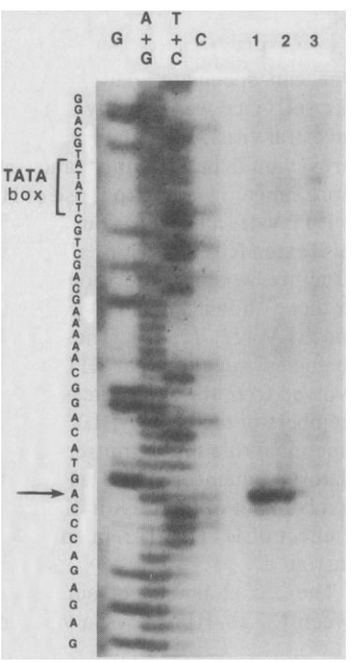

Fig. 3. S1 nuclease mapping of HTLV-III and the RNA initiation site. Procedure: 0.25 μg of HTLV-III virion RNA (lane 1), 5 μg of poly A(+) cellular RNA from HTLV-III infected H9 cells (lane 2), or uninfected H9 cells (lane 3) were hybridized to a Hind III–Hpa I fragment of clone HXB2 at 49°C for 3 hours in 40 m*M* Pipes, *p*H 6.4, 400 m*M* NaCl, 1 m*M* EDTA, and 80 percent formamide. The probe was labeled at the Hind III site with [γ-^{32}P]ATP and T4 polynucleotide kinase (*22*). Samples were then incubated with S1 nuclease (200 U/ml), 280 m*M* NaCl, 30 m*M* sodium acetate, *p*H 4.4, 4.5 m*M* zinc acetate, and denatured calf thymus DNA (20 μg/ml) at 45°C for 30 minutes (*23*). Samples were subjected to electrophoresis on an acrylamide gel. Samples of the same ^{32}P-labeled Hind III–Hpa I DNA fragment were subjected to electrophoresis in parallel after they had undergone sequence reactions (*22*). The (−) strand nucleotide sequence is shown to the left of the figure. Since the chemical cleavage reactions destroy the nucleotide at which the base-specific strand scission occurs (*22*), the actual sequence length of the major S_1 nuclease-protected fragment is 1 bp shorter than the corresponding position on the sequence ladder (arrow).

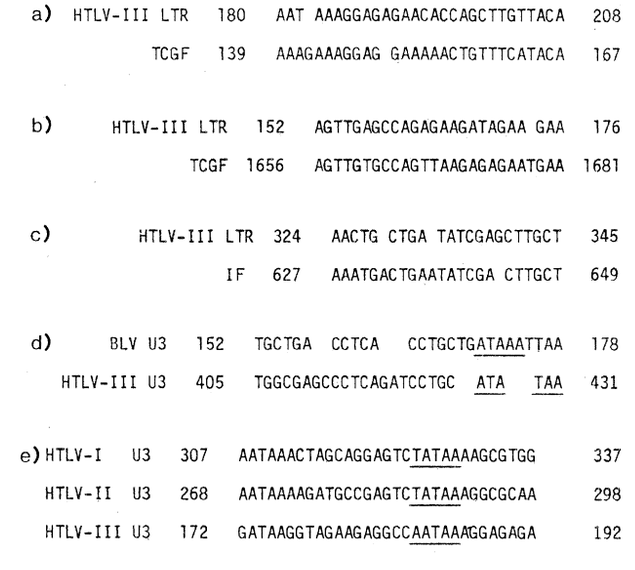

Fig. 4. (a) Sequence homology between the HTLV-III LTR and the TCGF gene. (b) Sequence homology between the HTLV-III LTR U3 region and the TCGF gene. (c) Sequence homology between the HTLV-III LTR U3 region and human γ-IF. (d) Sequence homology between HTLV-III U3 and BLV U3 regions. (e) Sequence homologies among the U3 regions of HTLV-I, HTLV-II, and HTLV-III. Gaps were introduced during complex analyses, to maximize the homologies (*35*).

immediately downstream from an enhancer-like sequence GTGGTTA.

Since the TCGF and γ-IF genes are expressed exclusively in T cells, and since the regions of homology are located downstream from their potential enhancer signals, the corresponding sequences in the HTLV-III LTR could play some role in host cell tropism or transcriptional regulation of this virus.

Some general features of the HTLV-III LTR are similar to those of HTLV-I, HTLV-II, and BLV. A 62 percent homology was found between HTLV-III U3 and BLV U3 sequences (*15*) that include the functional promoters of both viruses (Fig. 4d).

Homologies of 61 and 55 percent were also found between HTLV-III and HTLV-I and HTLV-II U3 sequences, respectively (*11, 12*). These sequences include the functional promoter signals of HTLV-I and HTLV-II and a promoter-like signal of HTLV-III U3 (AATAAA) (Fig. 4e) (position 527 in Fig. 2). One can speculate that this promoter-like sequence was actually utilized in an ancestral virus. If so, the R region of this virus would be extended and thus be more similar in length to those of HTLV-I, HTLV-II, and BLV. Furthermore, in this extended R region a stable loop structure would be positioned similarly to those shown for HTLV-I, HTLV-II, and BLV (*11, 12, 15*). These structural analogies suggest that HTLV-III has evolved from the same ancestor as other members of the HTLV/BLV family. The functional role of the transcriptional regulatory sequences in U3 as well as the involvement of the LTR in the cytopathic effect of HTLV-III remain to be demonstrated.

The recent finding of similarities between HTLV-III and visna virus in morphology, cytopathic potential, ability to infect brain tissue, and nucleotide sequence strongly suggests that these viruses are related (*7, 21*). It would be of interest to determine whether the LTR of visna is similar in structure to the LTR of HTLV-III.

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The Pollination of *Zygogynum* (Winteraceae) by a Moth, *Sabatinca* (Micropterigidae): An Ancient Association?

Abstract. *The primitive and vesselless angiosperm* Zygogynum *(Winteraceae), which is restricted to New Caledonia, is pollinated by a moth,* Sabatinca *(Micropterigidae). Fossil records of both the moth and the plant families extend to the Early Cretaceous. Adult* Sabatinca *have grinding mandibles and usually feed on the spores of ferns and on pollen. The insects use the flowers as mating sites and eat the pollen which is immersed in a dense pollenkitt. This mode of pollination in which flowers serve as mating and feeding stations with floral odors acting as cues may have been common in the early evolution of flowering plants.*

A complex flower-insect relation between two groups of archaic organisms in New Caledonia has been elucidated by field observations and chemical analysis of the flower odors. The life cycles of specific moths (*Sabatinca*) and trees (*Zygogynum*) are linked; aggregations of moths use the flowers as mating and feeding stations, and their activities result in pollination. This interaction is