

## New human and simian HIV-related retroviruses possess functional transactivator (*tat*) gene

Suresh K. Arya\*, Barbara Beaver\*, Linda Jagodzinski†, Barbara Ensoli\*, Phyllis J. Kanki‡, Jan Albert§, Eva-Maria Fenyo||, Gunnel Biberfeld§, Jean F. Zagury¶, Francoise Laure¶, Myron Essex†, Erling Norrby||, Flossie Wong-Staal\* & Robert C. Gallo\*

\* Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA

† Biotech Research Laboratories, Rockville, Maryland 20850, USA

‡ Department of Cancer Biology, Harvard School of Public Health, Boston, Massachusetts 02115, USA

§ The National Bacteriological Laboratory, Stockholm, Sweden

|| Department of Virology, Karolinska Institute, School of Medicine, Stockholm, Sweden

¶ Laboratoire de Biologie Cellulaire, University Pierre et Marie Curie, Paris, France

New human retroviruses antigenically related to HIV and even more closely to STLV-III have been recently isolated from individuals from some West African countries<sup>1-4</sup>. One of these viruses, HTLV-IV<sub>P</sub>, was reportedly isolated from lymphocytes of a healthy female prostitute<sup>1,2</sup>. Another isolate, LAV-2<sub>FG</sub>, was obtained from an AIDS patient and third, SBL-6669, from an individual with lymphadenopathy<sup>4</sup>. Current epidemiological studies indicate that some of these virus isolates cause immune deficiency whereas others may not or may be less efficient at inducing immune deficiency. Similarly, STLV-III apparently does not cause immune deficiency in its natural host, African green monkey<sup>5</sup>. A novel feature of HIV is the possession of a gene termed *tat*, which is implicated in its pathobiology<sup>6-12</sup>. We report here that, like HIV, HTLV-IV<sub>P</sub>, LAV-2<sub>FG</sub> (HIV-2) and SBL-6669, as well as STLV-III<sub>AGM</sub> possess the putative *tat* gene, irrespective of their pathogenic potential *in vivo*. Interestingly, HTLV-IV<sub>P</sub>/LAV-2<sub>FG</sub> long terminal repeat (LTR) is equally well transactivated by the HTLV-IV<sub>P</sub>/LAV-2<sub>FG</sub> and HTLV-III<sub>B</sub> *tat* function, HTLV-III<sub>B</sub> LTR responds better to its own *tat* function.

The human T-lymphotropic retroviruses (HTLVs) share many properties, including preferred tropism for T lymphocytes bearing the CD4 (T4) antigen, similar modes of transmission and, probably, African origin. They also differ significantly from each other, forming two distinct groups. HTLV-I<sup>13-16</sup>, a transforming virus, is the prototype of the first group. It causes adult T-cell leukaemia (ATL) and appears to be involved in B-cell malignancies and in central nervous system disease<sup>17,18</sup>. This group also includes HTLV-II<sup>19</sup> and variants of HTLV-I<sup>20</sup> which are associ-

ated with T-cell malignancies different from ATL. Human immune deficiency virus (HIV)<sup>21-24</sup>, also known as HTLV-III or lymphadenopathy virus (LAV), a non-transforming virus, is the prototype of the second group. It causes acquired immune deficiency syndrome (AIDS) and nervous system disorders. Recently, new members of this group of non-transforming human retroviruses were isolated from individuals from different countries of West Africa<sup>1-4</sup>. These isolates show major antigenic characteristics that distinguish them from HIV, although they retain many features in common<sup>1-4</sup>. Remarkably, counterparts of both groups of human retroviruses also exist in old-world subhuman primates. They have been termed simian T-lymphotropic virus-I and -III (STLV-I and -III). STLV-III, related to HIV and even more closely to HTLV-IV<sup>1-5</sup> does not appear to cause AIDS in its natural host, the African green monkey or related species.

To test if the new non-transforming human retroviruses contained a transactivating gene, we first cloned HTLV-IV<sub>P</sub>, LAV-2<sub>FG</sub> and STLV-III<sub>AGM</sub> LTRs by way of cDNA cloning. Several independent clones containing 3'-LTR and sequences extending upstream were characterized and sequenced. After this manuscript was submitted, the sequences of the LTRs of HIV-2<sup>25</sup>, HTLV-IV and STLV-III<sup>26</sup> and the complete sequence of the HIV-2 genome<sup>27</sup> were published. The sequences of our cDNA clones of these viruses are very similar or identical to the published reports. Differences of a few nucleotides, where observed, probably reflect strain differences. As with Kornfeld *et al.*<sup>25</sup>, we find the sequences of HTLV-IV and STLV-III to be more than 95% homologous. This may reflect inter-species transmission of the same or nearly identical virus(es). Cross-species transmission of retroviruses can occur under certain circumstances. For example, STLV-III infection of macaque monkeys may be a result of inter-species transmission from infected African green or mangabey monkeys kept in close proximity<sup>26-30</sup>. Similarly, HIV can be transmitted to chimpanzees<sup>31</sup> and HTLV-I to certain monkeys<sup>32</sup>. Kornfeld *et al.*<sup>25</sup> have also noted that HTLV-IV and STLV-III may not be independent virus isolates but result from transmission of the same virus to different cell cultures maintained in the laboratory. This is possible but it is equally possible that the transmission occurred in nature. Future studies with more human and simian virus isolates may clarify this issue.

For transactivation experiments, cloned HTLV-IV<sub>P</sub> DNAs (V17 and V8) and LAV-2<sub>FG</sub> DNA (LV-2) were placed upstream of the bacterial chloramphenicol acetyltransferase (CAT) gene carried on a vector lacking promoter-enhancer sequences (pSVOCat)<sup>28</sup> and the resulting plasmids were termed pV17Cat, pV8Cat and pLV2Cat. Two additional plasmids, pSV2Cat<sup>33</sup> and pC15Cat<sup>8,9</sup> respectively contained SV40 promoter-enhancer and HTLV-III 3'-LTR linked to the CAT gene. The plasmid DNAs were transfected into virus-infected and uninfected human HUT 78 cells by the DEAE-dextran protocol and transactivation of the LTR-linked CAT gene was measured by conversion of

Table 1 Transactivation of HTLV-IV, LAV-2 and HTLV-III LTRs in virus-infected cells

Plasmid	Regulatory elements	% Conversion of chloramphenicol to acetylated forms					
		HUT78*	HTLV-IV <sub>P</sub> /HT*	SBLV6669/HT*†	LAV-2 <sub>FG</sub> /HT*	STLV-III <sub>AGM</sub> /HT*	HTLV-III <sub>B</sub> /H9*
pSV2Cat	SV40	2.4±1.7	3.0±0.35	0.7±0.6	9.5±1.0	4.5±3.8	1.3±0.3
pSVOCat	None	0.12±0.01	0.12±0.01	0.14±0.03	0.16±0.02	0.15±0.03	0.14±0.01
pC15Cat	HTLV-III <sub>F</sub>	0.43±0.3	11.4±1.9	21.5±34.2	4.6	16.0±9.1	96±1.4
pV17Cat	HTLV-IV <sub>P</sub>	0.86±0.4	97.4±2.4	56.5±58.0	98.1±0.2	99.2	99±0.1
pV8Cat	HTLV <sub>P</sub>	1.3±0.8	98.7±0.2	56.7±39.0	98.3	98.0±2.0	98±0.8
pLV2Cat	LAV-2 <sub>FG</sub>	—	99.1	—	96.9	—	99.2
pV17Cat(nc)	HTLV-IV <sub>P</sub>	0.13±0.8	0.2±0.05	—	—	—	—

\* Mean and standard deviation for 2 to 4 transfection experiments, where shown.

† Relatively wide variation and/or lower values are probably related to the recent infection of the host cells by this virus. The general metabolism and viability of the culture and the proportion of infected cells in the population may fluctuate during early periods.

Fig. 1 A HTLV-III<sub>B</sub> α, uninfected, infected, SBLV-6669, STLV-III<sub>B</sub>, HTLV-III<sub>B</sub>, pSV2Cat, pC15Cat, (HTLV-IV<sub>P</sub>) and pV8Cat. A, transfection protocol as described in amphenicol ch

chloramphenicol in cellular described<sup>8</sup>, extensive c

The HTLV-III<sub>B</sub> enhancer sequence in uninfected HTLV-IV<sub>P</sub> (for example orientation lacking promoter activity uninfected w HTLV-III<sub>B</sub> to uninfected for LAV-2, contain a fa

TL. Human as HTLV-III... virus, is... red immune... disorders... transforming... from different... for antigenic... though they... counterparts... in old-world... simian T...). STLV-III...<sup>71-5</sup> does not... African green

oviruses con-... V-IV<sub>P</sub>, LAV... ning. Several... es extending... r this manu-... of HIV-2<sup>25</sup>... uence of the... f our cDNA... l to the pub-... ere observed... d *et al.*<sup>25</sup>, we... e more than... transmission... species trans-... cumstances... keys may be... African green...<sup>30</sup>. Similarly,

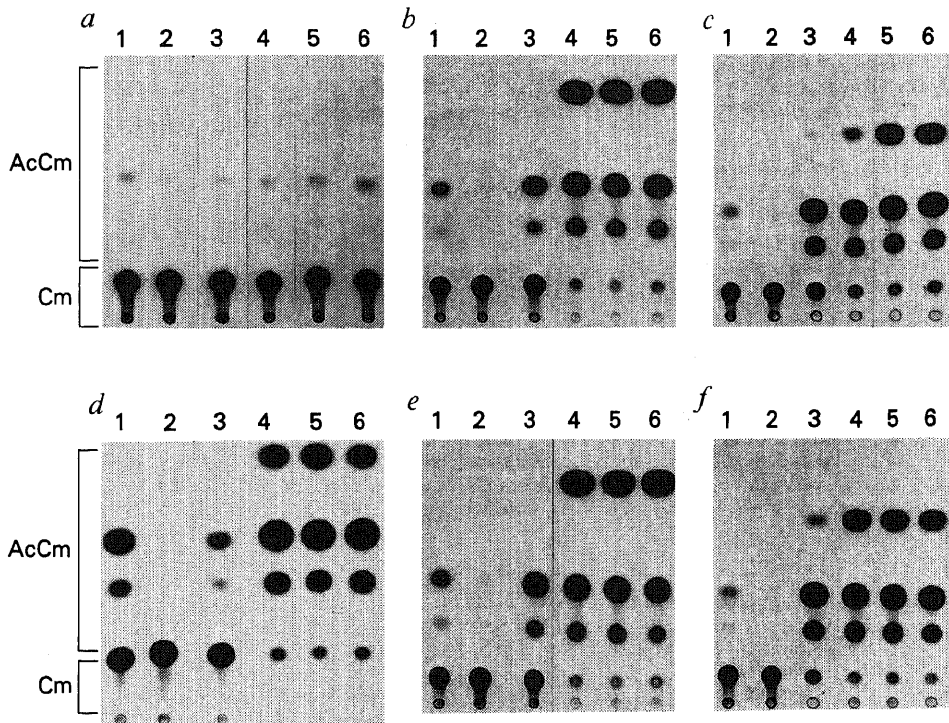
V-I to certain... HTLV-IV and... t result from... cell cultures... it is equally... ure studies... ify this issue... V-IV<sub>P</sub> DNAs... ced upstream... (CAT) gene... er sequences... ed pV17Cat... SV2Cat<sup>33</sup> and... enhancer and... asmid DNA... human HUT... activation of... onversion of

LV-III<sub>B</sub>/H9<sup>a</sup>

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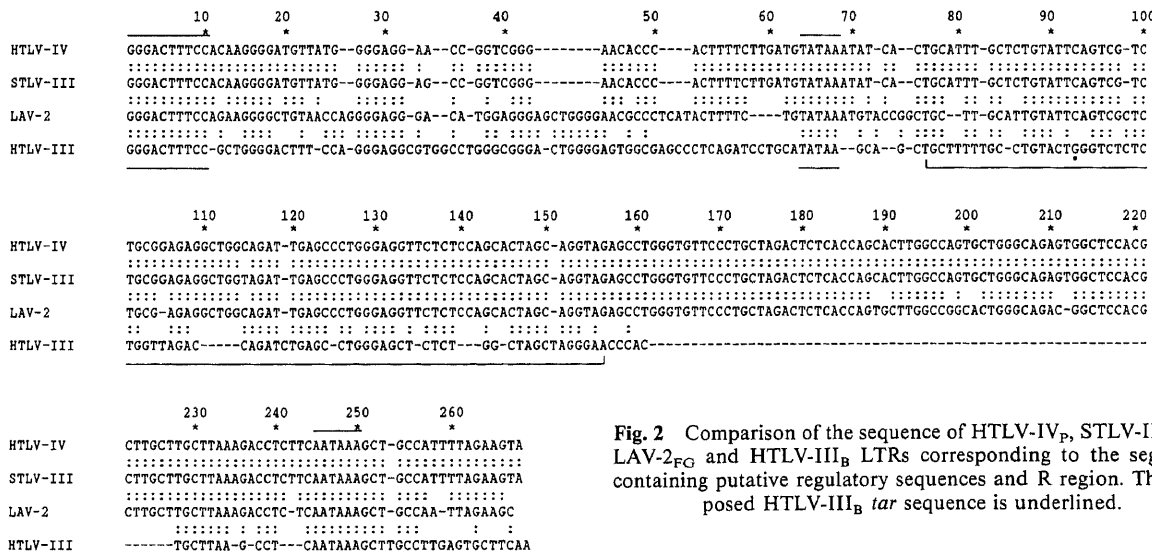
**Fig. 1** Activation of HTLV-IV<sub>P</sub> and HTLV-III<sub>B</sub> LTR-linked *cat* gene in *a*, uninfected HUT78 cells; *b*, cells infected with HTLV-IV<sub>P</sub>; *c*, with SBLV-6669; *d*, with LAV-2<sub>FG</sub>; *e*, with STLV-III<sub>AGM</sub> and *f*, with HTLV-III<sub>B</sub>. Lanes 1-6 are for pSV2Cat(SV40), pSVOCat(none), pC15Cat0HTLV-III<sub>A</sub>, pV17Cat(HTLV-IV<sub>P</sub>), pV17bCat(HTLV-IV<sub>P</sub>) and pV8Cat(HTLV-IV<sub>P</sub>), respectively. About 10 × 10<sup>6</sup> cells were transfected by the DEAE-dextran protocol and assays were performed as described before<sup>11,12</sup>. Cm, chloramphenicol; AcCm, acetylated chloramphenicol.



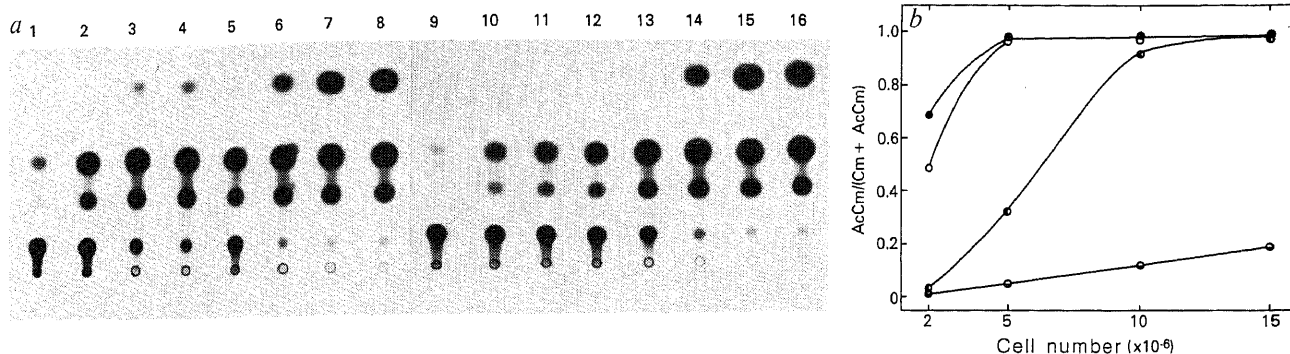
chloramphenicol to its acetylated forms catalysed by the enzyme in cellular extracts 44-48 h after transfection as previously described<sup>8,9</sup>. Representative results are shown in Fig. 1 and more extensive data are presented in Table 1.

The HTLV-IV<sub>P</sub> and LAV-2<sub>FG</sub> LTRs contain promoter-enhancer sequence elements, as expected. The CAT gene activity in uninfected HUT78 cells transfected with plasmid DNA with HTLV-IV<sub>P</sub> LTR linked in correct orientation to the CAT gene (for example, pV17Cat) was higher than that for the incorrect orientation (for example, pV17Cat-nc) or for the control plasmid lacking promoter-enhancer (pSVOCat; Fig. 1). The CAT gene activity under the control of HTLV-IV<sub>P</sub> LTR in HUT 78 cells infected with HTLV-IV<sub>P</sub>, LAV-2<sub>FG</sub> and SBL-6669 and with STLV-III<sub>AGM</sub> and HTLV-III<sub>B</sub>, was markedly elevated compared to uninfected cells (Fig. 1, Table 1). Similar results were obtained for LAV-2<sub>FG</sub> LTR. This indicates that the virus-infected cells contain a factor(s) which acts *in trans*, amplifying the expression

of the Cat gene linked to HTLV-IV<sub>P</sub> and LAV-2<sub>FG</sub> LTRs. HTLV-IV<sub>P</sub> and LAV-2<sub>FG</sub> LTRs responded to the transactivating factor in cells infected with all three of the HTLV-IV<sub>P</sub> related viruses as well as those infected with STLV-III<sub>AGM</sub> and HTLV-III<sub>B</sub>. These results suggest that (1) all new isolates (HTLV-IV<sub>P</sub>, LAV-2<sub>FG</sub> and SBL-6669) as well as STLV-III<sub>AGM</sub> contain a transactivating gene, (2) their putative transactivating genes are structurally and/or functionally homologous, (3) their LTRs are also structurally and/or functionally related and (4) the HTLV-IV<sub>P</sub> putative transactivating gene is related to the similar gene in HTLV-III<sub>B</sub>. The fact that HTLV-IV<sub>P</sub> and LAV-2<sub>FG</sub> LTRs are transactivated equally well in cells infected with HTLV-IV<sub>P</sub>, LAV-2<sub>FG</sub>, SBL-6669, STLV-III<sub>AGM</sub> and HTLV-III<sub>B</sub> whereas HTLV-III<sub>B</sub> LTR is more highly transactivated in cells infected with HTLV-III<sub>B</sub> than other viruses indicates that HTLV-IV<sub>P</sub>/LAV-2<sub>FG</sub>/SBL-6669/STLV-III<sub>AGM</sub> transactivating genes and LTRs are functionally more similar to each other than to



**Fig. 2** Comparison of the sequence of HTLV-IV<sub>P</sub>, STLV-III<sub>AGM</sub>, LAV-2<sub>FG</sub> and HTLV-III<sub>B</sub> LTRs corresponding to the segments containing putative regulatory sequences and R region. The proposed HTLV-III<sub>B</sub> *tar* sequence is underlined.



**Fig. 3** Comparative activation of HTLV-IV<sub>P</sub> and HTLV-III<sub>B</sub> LTRs by HTLV-IV<sub>P</sub> and HTLV-III<sub>B</sub> transactivating functions. Indicated number of virus infected cells were transfected with 10 µg of LTR-Cat plasmid DNAs and the cells were processed to obtain 100 µl of cytoplasmic extracts. Aliquots of 20 µl were used for cat assays<sup>11,12</sup> and incubation was for 60 min. Lanes 1-4, HTLV-III<sub>B</sub>-infected cells transfected with pC15Cat; lanes 5-8, HTLV-III<sub>B</sub>-infected cells transfected with pV17Cat; lane 9-12, HTLV-IV<sub>P</sub>-infected cells transfected with pC15Cat; lanes 13-16, HTLV-IV<sub>P</sub> infected cells transfected with pV17Cat.  $\blacktriangle$ , HTLV-IV<sub>P</sub>-infected cells transfected with pV17Cat;  $\circ$ , HTLV-III<sub>B</sub>-infected cells transfected with pV17Cat;  $\circ$ , HTLV-III<sub>B</sub>-infected cells transfected with pC15Cat;  $\circ$ , HTLV-IV<sub>P</sub>-infected cells transfected with pC15Cat.

HTLV-III<sub>B</sub>. A specific sequence (TAR) in HTLV-III<sub>B</sub> LTR responsible for optimal transactivation has been identified and localized between 6 base pairs (bp) and 76 bp downstream of the TATA box<sup>34-36</sup>. Such a specific sequence in HTLV-IV<sub>P</sub> and STLV-III<sub>AGM</sub> LTRs must lie within 62 bp upstream and 202 bp downstream of the TATA box. This is the only sequence contained in the fully active clone V8. Furthermore, the LTR sequences of HTLV-IV<sub>P</sub>/STLV-III<sub>AGM</sub>/LAV-2<sub>FG</sub> and HTLV-III<sub>B</sub> can be aligned such that they are about 65% homologous downstream of the TATA box in the proposed *tar* region of HTLV-III<sub>B</sub> (Fig. 2). The overall homology in the regulatory region of the LTRs of the new virus isolates is about 85-95%. Notably, the putative enhancer element (GGGACTTCC) is perfectly conserved and the potential SP1 binding sites are also highly conserved in HIV and HTLV-IV<sub>P</sub>/STLV-III<sub>AGM</sub>/LAV-2<sub>FG</sub>. In addition, the sequences downstream of the TATA box in HTLV-IV<sub>P</sub>/STLV-III<sub>AGM</sub>/LAV-2<sub>FG</sub> and HTLV-III<sub>B</sub> contain imperfect direct and indirect repeats which impart analogous structures to these LTRs and to the transcripts originating therein.

Interestingly, HTLV-IV<sub>P</sub> and LAV-2<sub>FG</sub> LTRs are equally well transactivated in cells infected with HTLV-IV<sub>P</sub>, LAV-2<sub>FG</sub> and HTLV-III<sub>B</sub>, whereas HTLV-III<sub>B</sub> is more transactivated in cells infected with HTLV-III<sub>B</sub> than with HTLV-IV<sub>P</sub> or LAV-2<sub>FG</sub> (Table 1). The differential response of HTLV-III<sub>B</sub> LTR in HTLV-III<sub>B</sub>- and HTLV-IV<sub>P</sub> or LAV-2<sub>FG</sub>-infected cells is unlikely to be related to lack of *tat* gene function in HTLV-IV<sub>P</sub>- or LAV-2<sub>FG</sub>-infected cells. HTLV-IV<sub>P</sub> and LAV-2<sub>FG</sub> LTRs are highly transactivated in HTLV-IV<sub>P</sub>- and LAV-2<sub>FG</sub>-infected cells. Furthermore, when the source of the *tat* gene function is varied by using different numbers of cells for transfection, the differential response of HTLV-III<sub>B</sub> LTR is maintained (Fig. 3). It may be that the HTLV-IV<sub>P</sub>/LAV-2<sub>FG</sub> LTR transactivator interaction has a broader specificity than HTLV-III<sub>B</sub> LTR transactivator interaction. Additionally, promoter-enhancer elements of HTLV-IV<sub>P</sub>/LAV-2<sub>FG</sub> may be stronger than such elements in HTLV-III<sub>B</sub>, allowing optimal transactivation even with heterologous HTLV-III<sub>B</sub> transactivator.

Some results comparing transactivation of the HIV-2 (LAV-2) and HIV-1 LTRs by the homologous and heterologous *tat* functions were recently reported by Guyader *et al.*<sup>27</sup> They also found that whereas HIV-2 LTR responded highly to the *tat* function of HIV-1 as well as to its own *tat* function, HIV-1 responded better to its own *tat* function.

The cells infected with all four virus isolates of the West African subgroup (HTLV-IV, LAV-2, SBL-6669 and STLV-III) contain a factor that activates their LTRs *in trans*, indicating that these viruses possess a functional transactivating gene. The transactivating gene of HIV has been implicated in its

pathobiology. As HTLV-IV<sub>P</sub> and STLV-III<sub>AGM</sub> may not always be pathogenic *in vivo*, either their transactivating genes do not function *in vivo*, an unlikely possibility, or this gene function is not sufficient for pathogenicity. Host factors or other viral factors directly or indirectly may modulate the potential pathogenic role of the transactivating genes of these viruses. The interaction between HTLV-IV<sub>P</sub>/STLV-III<sub>AGM</sub>/LAV-2 (HIV-2) LTRs and the *tat* gene product apparently has broader specificity than the HTLV-III (HIV) LTR: *tat* interaction.

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Shashidhar N & Peter A. Ko

Department of P San Francisco, C

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\* Present addresses: D 92037, USA (U.C.S.); D 02138, USA (P.A.B.).

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