biomass burning (*11*). An increased CO concentration on the other hand was suggested as a reason for a decrease of OH radicals in the atmosphere (*12*). A decrease of OH radicals, the main sink for CH_4 (*13*), is therefore leading to an increase of the CH_4 concentration (*5*). The rapid increase of the CH_4 concentration may therefore be caused in part by an increase of the CO concentration.

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13. The question arises whether OH radicals could be responsible for a depletion of CH_4 in the air bubbles after enclosure. A possible source for OH radicals is H_2O_2 dissolved in the ice and diffusing slowly into the air bubbles. Different concentrations of H_2O_2 in ice from different locations have been measured by A. Neftel, P. Jacob, and D. Klockow [*Nature (London)* **311**, 43 (1984)]. The influence of such a reaction in natural ice would be small. Whether the suggested reaction occurs at all in natural ice is not yet known.

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Transcription of Novel Open Reading Frames of AIDS Retrovirus During Infection of Lymphocytes

Abstract. *The retrovirus frequently isolated from patients with the acquired immune deficiency syndrome (AIDS) has two novel open reading frames previously designated "A" and "B." The "A" region was found to be specifically expressed as polyadenylated RNA's of 5.5 and 5.0 kilobases in infected cells. The "B" region was expressed as 1.8- to 2.0-kilobase RNA species. Additional full-length and spliced messenger RNA's of the* env *region were also identified.*

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A novel human retrovirus has been isolated from patients with acquired immune deficiency syndrome (AIDS) and the AIDS-related complex (ARC) (*1*). In vitro, this retrovirus is cytopathic for human T-cells of the OKT4/Leu-3 phenotype, the same cells that are selectively depleted in AIDS patients. This effect on lymphocytes, coupled with seroepidemiologic data linking infection by the AIDS retrovirus (AIDS RV) with the occurrence of disease, has implicated the AIDS RV as the etiologic agent of AIDS. A crucial question in understanding the pathogenesis of AIDS is the mechanism by which the AIDS RV specifically infects and kills lymphocytes. In addition to the *gag*, *pol*, and *env* genes, the AIDS RV genome contains two unique open reading frames (*2, 3*) which we have previously called "A" and "B" (*4*). In this report we show that both the "A" and "B" genes are transcribed as spliced subgenomic RNA's in cells infected by the AIDS RV.

We have used infected normal human lymphocytes stimulated by phytohemagglutinin (PHA), as well as a continuous human T-cell line, A3.01 (*5*), to study the transcription of viral RNA during cytolytic infection. AIDS RV infection of A3.01 cells mimics that seen in stimulated lymphocytes; reverse transcriptase (RT) activity can be detected in culture supernatants several days after infection, and cell death follows shortly thereafter. We have also examined AIDS RV transcription in the continuous virus producer line H9 (*6*).

Polyadenylated [poly(A)$^+$] RNA was prepared from acutely infected PHA-stimulated lymphocytes or A3.01 cells and from virus producing H9 cells. Infected PHA-stimulated lymphocytes and A3.01 cells were harvested after the onset of viral cytopathic effect (syncytia formation) but before the appearance of RT activity. Infected cell RNA preparations were then examined by Northern blot hybridization with the use of molecularly cloned segments of the AIDS RV DNA as probes (Fig. 1). The long terminal repeat (LTR) (probe 1), *gag* (probe 2), *pol* (probe 3), *pol*-"A" (probe 4), and *env* (probe 5) DNA segments were isolated by restriction enzyme cleavage of plasmid subclones of the lymphadenopathy virus (LAV) proviral DNA (*7*). Two 30-bp oligonucleotide probes, specific for the novel open reading frames of the AIDS RV, were synthesized according to the published sequences of LAV and ARV-2 (*2*). In this report we use a nucleotide numbering system based on the sequence of a complete copy of LAV proviral DNA. To convert nucleotide positions to those of the AIDS RV RNA genome, one should subtract the number of nucleotides present in the U3 LTR: 456 bp for LAV, 453 bp for HTLV-III and ARV-2 (*2, 3*). The "A" region oligomer (5562–5591) was a perfect match (30/30 bp) with both the LAV and ARV-2 sequences. The synthetic "B" region probe (8892–8921) was a 30/30-bp match with the LAV sequence and a 29/30-bp match with ARV-2.

Figure 2 shows Northern blot hybridizations of poly(A)$^+$ RNA from cells infected with AIDS RV. In these experiments, stimulated human lymphocytes (lanes a to c) or A3.01 cells (lanes d to k) were infected with virus isolated from an AIDS patient living in New York. This isolate, NY-5, has biological and biochemical properties similar to other AIDS RV's; a molecular clone of NY-5 proviral DNA contains a number of restriction sites present in several other viral isolates (*8*). Since all retroviral messenger RNA's (mRNA's) have LTR sequences at their 5′ and 3′ ends, the complete array of AIDS RV transcripts present in an infected cell should hybridize to an LTR probe (probe 1). As shown in Fig. 2, lane a, five discrete LTR-reactive bands were present in infected stimulated lymphocytes. These included the full-length, 9.1-kb viral genomic RNA and comigrating *gag*/*pol* mRNA's; four subgenomic mRNA species of 5.5, 5.0, 4.3, and 1.8 to 2.0 kb in size were also seen. Similar LTR-reactive transcripts were also observed in A3.01 cells infected with NY-5 (lane d). No hybridization of the LTR probe to RNA prepared from uninfected stimulated lymphocytes

(lane c) or uninfected A3.01 cells (lane k) could be demonstrated.

Retroviral mRNA's are generated by splicing events in which 5′ LTR sequences are joined to a splice acceptor (or acceptors) that precede a retroviral gene. Since the viral genome contains a single functional polyadenylation signal and site, spliced retroviral mRNA terminates with 3′ LTR sequences. Consequently, hybridization probes derived from the 3′ portion of a retroviral genome generally hybridize to mRNA's whose splice acceptors are 5′ to the probe irrespective of whether the reactive RNA segment is translated or not. It should be noted that the splicing patterns used by the AIDS RV, as deduced by complementary DNA clones (*3, 9*), exhibit considerable complexity resulting in several doubly spliced mRNA species. Thus Northern blot analyses may not allow complete characterization of these complex species of AIDS mRNA. As shown in Fig. 2, lane e, DNA from the *gag* region (probe 2) hybridized to a 9.1-kb RNA species; this band includes the unspliced genomic RNA and putative *gag* mRNA. The *pol* probe (probe 3) also reacted with the 9.1-kb RNA (lane f). A minor RNA species of 7.4 kb also hybridized to the *pol* probe and could represent a low abundance *pol* mRNA. When a DNA segment encompassing the 3′ end of *pol* and 5′ portion of the "A" region (probe 4) was annealed to infected cell RNA (lane g), two additional species of 5.5 and 5.0 kb were detected. These two RNA's also hybridized to the "A" region 30-bp oligonucleotide probe (lanes b and h), thus confirming that they contained "A" gene sequences. These results demonstrate that the 5.5- and 5.0-kb RNA's were present in stimulated human lymphocytes and A3.01 cells undergoing cytolytic infection. The 5.5- and 5.0-kb RNA's were also detected in A3.01 cells infected with LAV (not shown) and are therefore not an artifact of the NY-5 infection. A labeled *env* region segment (probe 5) annealed to a 4.3-kb mRNA (lane i) as well as to the 9.1, 5.5, and 5.0 kb species detected previously; the reactivity of the *env* probe with the latter RNA's is due to the presence of 3′ untranslated sequences. Finally, a synthetic oligonucleotide derived from the "B" region reacted with all of the mRNA's detected by 5′ viral probes but, in addition, hybridized to a 1.8- to 2.0-kb RNA band (lane j).

In previous analyses of AIDS RV transcription, investigators have examined viral mRNA expression in the continuous HTLV-III producer cell lines H9 (*3*) and H4 (*9*), both of which contain 9.4-, 4.2-, and 1.8- to 2.0-kb poly(A)$^+$ RNA's. No 5.0- or 5.5-kb "A" region RNA's were detected in either H9 or H4 cells producing HTLV-III (*3, 9*). Since the two "A" region AIDS RV RNA's were present in cytolytically infected lymphocytes, we wondered whether the 5.0- and 5.5-kb viral RNA species might encode cytolytic functions. To test this hypothesis, we prepared poly(A)$^+$ RNA from HTLV-III–producing H9 cells (*6*). This RNA was hybridized to subgenomic segments of AIDS RV proviral DNA (lanes l and m). The LTR probe (probe 1) hybridized to the same five RNA species in H9 cells (lane 1) as observed in infected lymphocytes (lane a) and A3.01 cells (lane d). The 5.5- and 5.0-kb RNA's present in H9 cells also hybridized to the synthetic "A" region probe (lane m) as well as to the *env* region DNA (not shown).

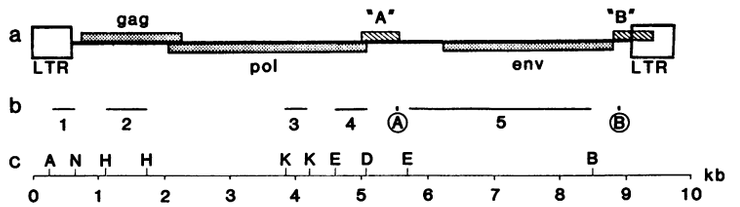

Fig. 1. AIDS retrovirus DNA probes. (A) Genetic organization of the AIDS retrovirus. (B) Probes used for Northern hybridizations. Probes 1 to 5 were subcloned from molecularly cloned integrated LAV provirus (*7*). These DNA fragments were purified from agarose gels after restriction enzyme digestion. Synthetic oligonucleotides complementary to "A" (5′ GTGGCCCTTGGTCTTCTGGGGCTTGTTCCA) and "B" (5′ CCAGGTCTCGAGATGCTGCTCCCACCCCAT) region mRNA's are so labeled. (C) Restriction endonuclease sites defining probes 1 to 5: A, Ava I; N, Nar I; H, Hind III; K, Kpn I; E, Eco RI; D, Nde I; B, Bam HI.

At present we cannot explain the discrepant results regarding the 5.5- and 5.0-kb AIDS RV mRNA's in virus-producing H9 cells. The infected H9 cells that we used were received at the Centers for Disease Control, Atlanta, Georgia, from R. C. Gallo's laboratory and have been grown continuously since 2 August 1984. These cells were propagated as described by Popovic *et al.* (*6*), with the exception that interleukin-2 (5 percent) was added to the medium 72 hours before they were harvested. The cells produced virus continuously, as monitored by supernatant RT activity, and exhibited some syncytia formation

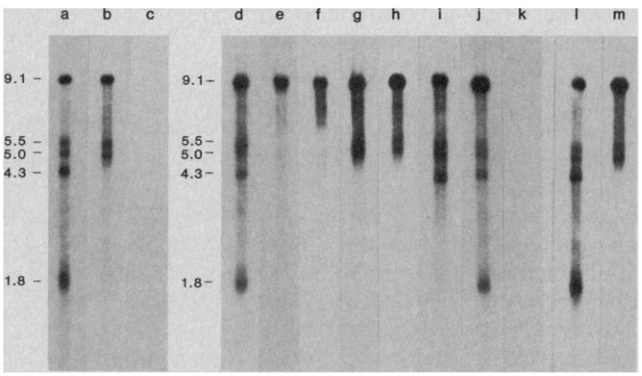

Fig. 2. Northern hybridization analysis of AIDS retrovirus RNA's. The RNA's were extracted by the guanidinium thiocyanate procedure (12). Poly(A)$^+$ RNA's (*13*) were subjected to electrophoresis on 1 percent formaldehyde agarose gels (*14*), transferred to nitrocellulose membranes (*15*), and hybridized as previously described for subcloned probes (*16*). Hybridization of RNA's with synthetic oligonucleotides was carried out in 50 m*M* tris, *p*H 8, sodium pyrophosphate (0.5 mg/ml), 1*M* NaCl, 10 percent dextran sulfate, 1 percent sodium dodecyl sulfate (SDS), and yeast RNA (1 mg/ml) (*17*). Subcloned probes were labeled with ^{32}P to specific activities of 5×10^8 to 10×10^8 cpm/μg by priming DNA synthesis with random hexamers (*18*). Synthetic probes were radiolabeled with ^{32}P to specific activities of 3×10^8 to 4×10^8 cpm/μg by incubation with T4 polynucleotide kinase. Poly(A)$^+$ RNA's were analyzed from AIDS RV-infected stimulated lymphocytes (0.8 μg per lane in lanes a and b), infected A3.01 cells (1 μg per lane in lanes d to j), uninfected A3.01 cells (2 μg in lane k), and infected H9 cells (2 μg per lane in lanes l and m). Total RNA was examined from uninfected stimulated lymphocytes (2 μg in lane c). Radiolabeled DNA probes (see Fig. 1) used were: probe 1 (lanes a, c, d, k, and l), probe 2 (lane e), probe 3 (lane f), probe 4 (lane g), probe 5 (lane i), probe A (lanes b, h, and m), and probe B (lane j). Filters hybridized with subcloned probes were washed in 0.1× standard saline citrate (SSC) with 0.1 percent SDS at 50°C, while those hybridized with synthetic oligonucleotides were washed in 1× SSC, 1 percent SDS, and sodium pyrophosphate (0.5 mg/ml) at 45°C. Blots were then exposed to x-ray film at −70°C for 30 to 90 minutes (lanes d to j, l, m), 12 hours (lanes a, b), or 3 days (lanes c, k). Sizes for AIDS RV RNA species in kilobases were determined by comparison with the 8.2- and 3.0-kb mRNA's of murine leukemia viruses subjected to electrophoresis on the same gels.

with no more cell death than previously reported (*6*). Since the H9 line was derived by infection of target HT cells with pooled AIDS patient material (*6*), different proviruses with variant transcriptional patterns might be expressed in different H9 populations. Alternatively, the results we report could reflect differences in RNA analysis. Our poly(A)$^+$ RNA's have been subjected to electrophoresis through long 1 percent agarose gels (migration of bromophenol blue dye = 18 cm) at 40 to 50 V to allow clear separation of large RNA species such as the 5.5- and 5.0-kb transcripts. RNA's of this size are not clearly resolved in the analysis of H4 cells (*9*).

Although the Northern blot hybridizations shown above demonstrate that the 5.5- and 5.0-kb AIDS RV RNA's contain LTR, "A," *env*, and "B" region sequences, such an analysis can only approximate the actual mRNA structure. For example, the 5.5- and 5.0-kb mRNA's hybridize to probe 4 DNA (Figs. 1 and 2) but not to probe 3. This implies that the splice acceptors for these mRNA's must lie between the 3′ end of probe 3 (position 4156) and the 3′ end of probe 4 (position 5134). In preliminary S1-nuclease experiments (*10*), we have identified a major splice acceptor 5′ to the "A" region at 4914 bp that could generate an mRNA of approximately 5.0 kb that would be in the proper reading frame for translation of "A" sequences. In addition, we have identified a second splice acceptor site at 4542 bp which could serve as an acceptor for the 5.5-kb RNA. Complementary DNA clones of these novel mRNA's will allow detailed study of their structure. The potential function of an "A" region gene product (or products) remains obscure. The *trans*-activating protein of the AIDS RV has recently been shown to be encoded by a doubly spliced mRNA (*9*) that contains 215 bp of coding sequence entirely 3′ to the "A" open reading frame (positions 5376 to 5590). Furthermore, deletion of the "A" coding region does not abolish the *trans*-activation property of the AIDS RV (*11*). Thus "A" does not encode the *trans*-activating property nor does it specify any known viral structural protein. Site-specific mutagenesis of "A" region sequences as well as expression of cDNA clones may elucidate the function of this novel retroviral gene.

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The t(14;18) Chromosome Translocations Involved in B-Cell Neoplasms Result from Mistakes in VDJ Joining

Abstract. *In this study, the joining sequences between chromosomes 14 and 18 on the 14q$^+$ chromosomes of a patient with pre–B-cell leukemia and four patients with follicular lymphoma carrying a t(14;18) chromosome translocation were analyzed. In each case, the involved segment of chromosome 18 has recombined with the immunoglobulin heavy-chain joining segment (J_H) on chromosome 14. The sites of the recombination on chromosome 14 are located close to the 5′ end of the involved J_H segment, where the diversity (D) regions are rearranged with the J_H segments in the production of active heavy-chain genes. As extraneous nucleotides (N regions) were observed at joining sites and specific signal-like sequences were detected on chromosome 18 in close proximity to the breakpoints, it is concluded that the t(14;18) chromosome translocation is the result of a mistake during the process of VDJ joining at the pre–B-cell stage of differentiation. The putative recombinase joins separated DNA segments on two different chromosomes instead of joining separated segments on the same chromosome, causing a t(14;18) chromosome translocation in the involved B cells.*

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Reciprocal chromosomal translocations involving the heavy-chain locus on chromosome 14 (*1*, *2*) have been observed in human B-cell neoplasms (*3*, *4*) and in Burkitt lymphomas (*5*). In diffuse B-cell lymphomas and in chronic lymphocytic leukemias of the B-cell type carrying the t(11;14)(q13;q32) chromosome translocation (*4*, *6*), the *bcl*-1 locus, normally located at band q13 of chromosome 11, translocates to the heavy-chain locus on chromosome 14 (*7*). In follicular lymphomas with the t(14;18) translocation, the *bcl*-2 gene (which is normally located at band q21 of chromosome 18) translocates to the heavy-chain locus (*8*, *9*). The translocation results in enhanced expression of the *bcl*-2 gene (*9*). By analyzing the joining between chromosomes 11 and 14 on the 14q$^+$ chromosome of two independent cases of chronic lymphocytic leukemia of the B-cell type with the t(11;14) chromosome translocation, we discovered that the breakpoints on chromosome 14 involved the J_H (J, joining; H, heavy) region at the sites where D (diversity) segments recombine with J_H DNA segments to generate a productively rearranged heavy-chain gene (*10*).

In this study, we have characterized the chromosome breakpoints in a pre–B-cell leukemia (380) (*8*, *11*) and in four independent follicular lymphomas carrying a t(14;18) chromosome translocation to investigate the molecular mechanisms involved in the t(14;18) chromosome translocation. We have previously described the cloning of the joining between chromosomes 14 and 18 in a pre–B-cell leukemia carrying a t(14;18) chromosome translocation (*8*), and the con-