

Characterization of a Novel Simian Immunodeficiency Virus (SIVmonNG1) Genome Sequence from a Mona Monkey (*Cercopithecus mona*)

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A novel simian immunodeficiency virus (SIV) sequence has been recovered from RNA extracted from the serum of a mona monkey (*Cercopithecus mona*) wild born in Nigeria. The sequence was obtained by using novel generic (degenerate) PCR primers and spans from two-thirds into the *gag* gene to the 3' poly(A) tail of the SIVmonNG1 RNA genome. Analysis of the open reading frames revealed that the SIVmonNG1 genome codes for a Vpu protein, in addition to Gag, Pol, Vif, Vpr, Tat, Rev, Env, and Nef proteins. Previously, only lentiviruses infecting humans (human immunodeficiency virus type 1 [HIV-1]) and chimpanzees (SIVcpz) were known to have a *vpu* gene; more recently, this has also been found in SIVgsn from *Cercopithecus nictitans*. Overall, SIVmonNG1 most closely resembles SIVgsn: the *env* gene sequence groups with HIV-1/SIVcpz *env* sequences, whereas the *pol* gene sequence clusters closely with the *pol* sequence of SIVsyk from *Cercopithecus albogaris*. By bootscanning and similarity plotting, the first half of *pol* resembles SIVsyk, whereas the latter part is closer to SIVcol from *Colobus guereza*. The similarities between the complex mosaic genomes of SIVmonNG1 and SIVgsn are consistent with a shared or common lineage. These data further highlight the intricate nature of the relationships between the SIVs from different primate species and will be helpful for unraveling these associations.

It is becoming increasingly apparent that many African primates are infected with lentiviruses (33). These simian immunodeficiency viruses (SIVs) are of complex evolutionary origin (40, 41). In earlier studies, primate lentiviruses were recovered from chimpanzees (SIVcpz, *Pan troglodytes*) (13, 21), sooty mangabeys (SIVsm, *Cercocebus atys*) (20); African green monkeys (SIVagm, *Chlorocebus aethiops*) (1, 19, 23, 25), Sykes monkeys (SIVsyk, *Cercopithecus albogaris*) (11, 18), and mandrills (SIVmnd-1, *Mandrillus sphinx*) (49). More recently, such viruses have been found and characterized in a wide range of primates, including the red-capped mangabey (SIVrcm, *Cercocebus torquatus torquatus*) (5, 15), guereza colobus monkey (SIVcol, *Colobus guereza*) (9), L'Hoest's monkey (SIVlhoest, *Cercopithecus lhoesti*) (17), sun-tailed monkey (SIVsun, *Cercopithecus solatus*) (3), talapoin monkey (*Myopithecus talapoin*) (30), mandrills (SIVmnd-2) (43), and drill monkey (SIVdrl, *Mandrillus leucophaeus*) (8). Most recently, a novel SIV has been found in the greater spot-nosed monkey (SIVgsn, *Cercopithecus nictitans*) (10). Some primate lentiviruses are able to cross the species barriers and give rise to new strains: SIVcpz has infected humans more than once to give human immunodeficiency virus type 1 (HIV-1) (13, 21, 22, 34, 50), and SIVsm has infected humans and macaques to yield HIV-2 and SIVmac, respectively (16, 20, 29). SIVagm has been recovered from baboons (*Papio* spp.) and a patas monkey (*Erythrocebus patas*), presumably as a result of natural transmissions in the wild (6, 24, 51). Some SIVs have mosaic or recombinant ge-

nomes, indicating that they have arisen as a result of infections with more than one distinct SIV. Examples are SIVagmSAB from the Sabaeus monkey (23), SIVrcm (5, 15), SIVdrl (8) (J. P. Clewley, unpublished observations), SIVmnd-2 (43), and SIVgsn (10). Evidence of cross-species transmission and multiple infections with SIVs is consistent with the suggestion that the primate lentiviruses originated and evolved in monkeys of the *Cercopithecus* genus (3, 17, 25).

The SIVs can be classified into distinct lineages based upon their complete genome sequence relationships (4). To date, six such lineages are recognized: SIVcpz/HIV-1, SIVsm/HIV-2/SIVmac, SIVlhoest/SIVsun/SIVmnd-1, SIVagm, SIVsyk, and SIVcol. New lineages are likely to be defined when the full sequences of several as yet only partially characterized genomes become available (33). The SIVs can also be classified into three groups based on the organization of the accessory genes in their genomes (4). All simian lentiviruses possess *vif*, *vpr*, and *nef* accessory genes and *tat* and *rev* regulatory genes but, until recently, only the SIVcpz and HIV-1 lineage viruses were known to have a *vpu* gene (4, 5, 27, 46). SIVgsn from the greater spot-nosed monkey has now also been shown to have a *vpu* gene (10). The SIVsm/HIV-2 lineage viruses, including SIVrcm and SIVmnd-2, do not have a *vpu* but do have a *vpx* gene (5, 43). The Vpu protein and gene have both been extensively studied in the expectation that they might shed light on the unique pathogenesis of HIV-1. For example, although the Vpu protein is not essential for viral replication, removal of the *vpu* gene leads to a decrease in pathogenicity (44). The origin of *vpu* is unclear, but it may have arisen to enhance viral release as a result of increased affinity of the Env protein for the CD4 receptor (45).

In the present study, we have recovered a genome sequence (SIVmonNG1) from lentivirus RNA extracted from the serum

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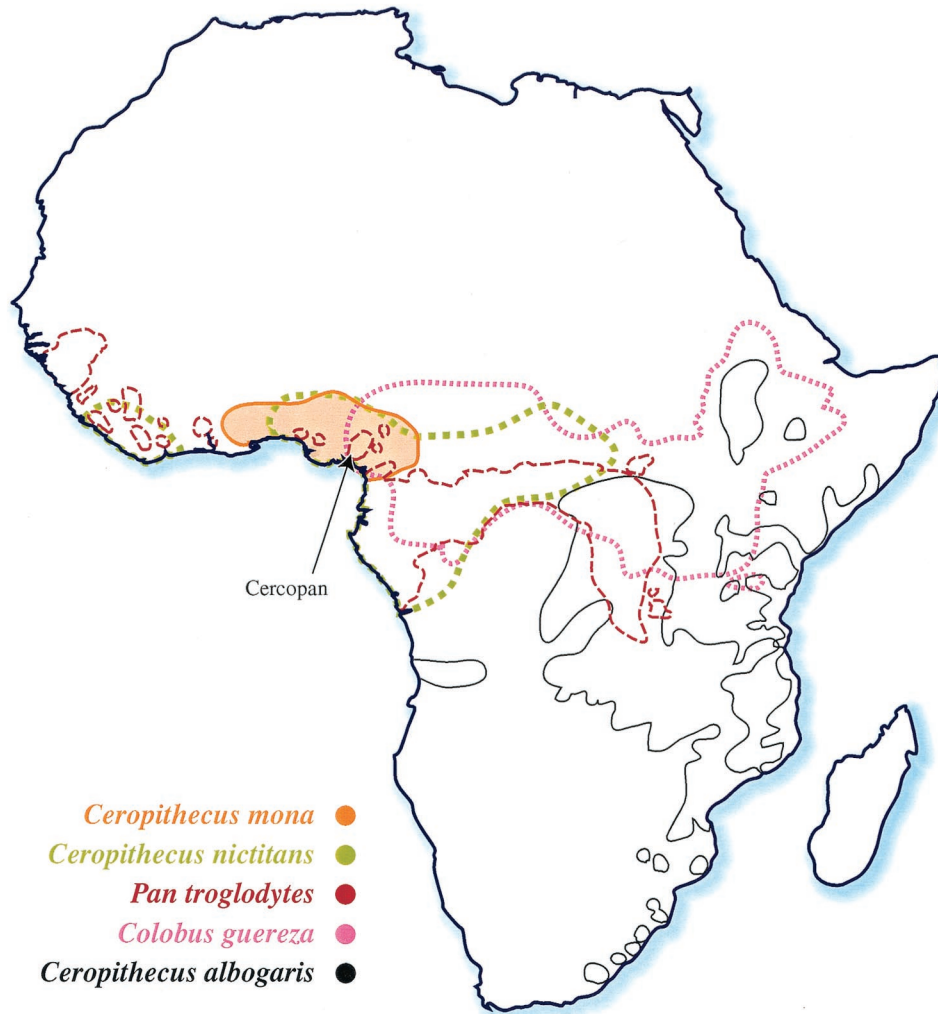


FIG. 1. Map of Africa showing the natural ranges of the mona monkey, *Cercopithecus mona*, the greater spot-nosed monkey, *Cercopithecus nictitans*, the chimpanzee, *Pan troglodytes*, the Sykes monkey, *Cercopithecus albogaris*, and the colobus monkey, *Colobus guereza*. The data were obtained from the study by Rowe (36) and from an online source (<http://gorilla.bio.uniroma.it/>). The approximate location of the Cercopan Project in Nigeria is arrowed.

of a mona monkey (*Cercopithecus mona*). This forest-dwelling guenon has a range from Ghana to Cameroon in western Africa (Fig. 1) (36). There are about 26 species of guenons, of which seven forest species, including the mona, live in southern Nigeria. They are endangered through habitat loss and hunting, and the Cercopan Project, located in southeastern Nigeria, is a nongovernmental organization dedicated to their conservation, rescue, and rehabilitation (<http://www.cercopan.org/>). A partial *pol* SIV sequence from a mona monkey from Cameroon has recently been described (33). Here, we show that the SIVmonNG1 genome, like SIVgsn, has a *vpu* gene, and that its *env* gene is related to SIVcpz sequences and its *pol* gene to SIVsyk and SIVcol.

MATERIALS AND METHODS

Specimens and serological testing. Serum samples were obtained from two mona monkeys during quarantine testing at Cercopan. These monkeys were obtained via the bush meat trade and thus were wild born; they were either confiscated by appropriate wildlife officials and subsequently donated to Cercopan

or donated by their owners who had purchased them from hunters. In the majority of cases the monkeys at Cercopan become captive at a very young age (6 months or less) and arrived at the sanctuary soon after. The sera were tested at the Central Public Health Laboratory for antibodies cross-reacting with HIV antigens by Innogenetics and Organon Teknika enzyme immunoassays (EIAs). A positive result in these tests was confirmed by using the Genelabs Western blot test.

RNA extraction, PCR amplification, cloning, and sequencing. RNA was extracted from serum by a modified guanidinium isothiocyanate-silica method (7) and converted to cDNA by reverse transcription by using Expand reverse transcriptase (RT; Roche Diagnostics, Lewes, United Kingdom). This was PCR amplified by using Expand High Fidelity or Long Template PCR reagents (Roche). Specific primers were used at a concentration of 300 nM, and degenerate primers were used at a concentration of 1,000 nM. Thermocycling conditions were modified appropriately for the target size: typically the first-round amplifications were 94°C for 2 min, followed by 10 cycles of 94°C for 15 s, 50°C for 30 s, and 68°C for 4.5 to 7 min, followed by a further 25 cycles, with an additional 5-s extension per cycle and a 4°C hold at the end. A total of 1.5 μ l of the primary PCR was added to a secondary PCR mixture and typically cycled at 94°C for 2 min, followed by 4 to 8 cycles of 94°C for 15 s, 42 to 50°C for 30 s, and 68°C (or 72°C for Expand High Fidelity) for 2 to 7 min, followed by a further 27 to 31 cycles, with an additional 5-s extension per cycle and a 4°C hold. On

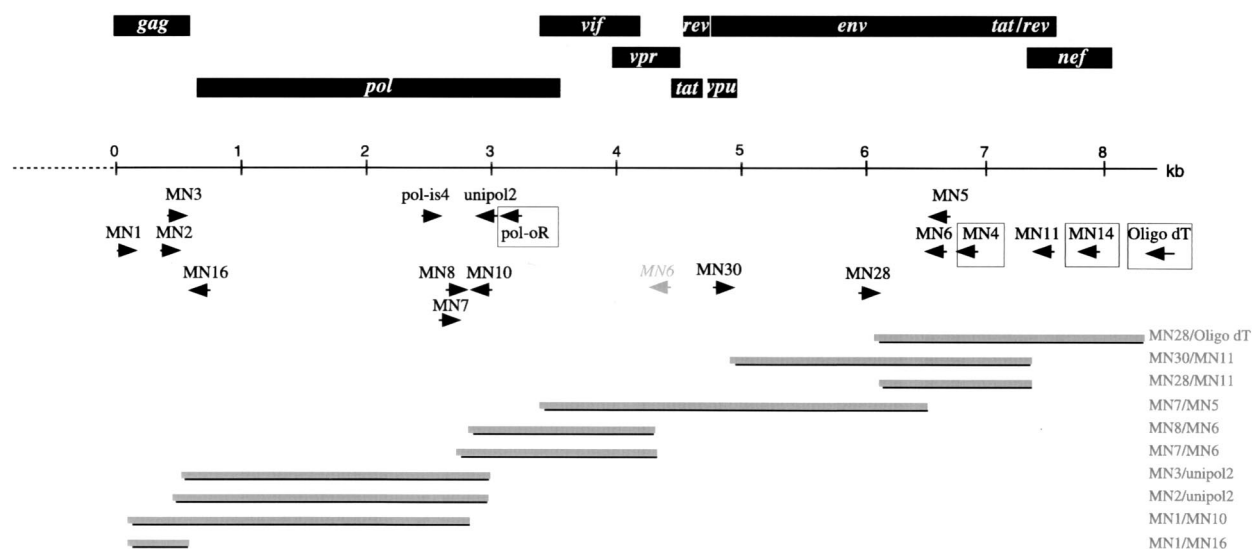


FIG. 2. Genome organization and PCR cloning strategy for SIVmonNG1 RNA. The identified ORFs are indicated by rectangular boxes above the scale bar. The alternative binding site of MN6 is shown in italics. The primers used for PCR are shown below the scale bar; primers used for cDNA synthesis are boxed. The position of the clones is shown by solid bars, and the primers used to obtain them are indicated on the right.

occasion, AccuPrime *Taq* polymerase (Invitrogen, Paisley, United Kingdom) was used for nested-PCR amplifications with previously described touchdown conditions (8). A 3' RACE (rapid amplification of cDNA ends) kit (Invitrogen) was used to recover sequences from the poly(A) tail to the *nef* gene. PCR products were cloned with TOPO XL kits (Invitrogen) and plasmids purified with Qiagen kits (Qiagen, Crawley, United Kingdom). Sequences were generated by using a capillary sequencer (CEQ 2000XL DNA analysis system; Beckman Coulter, High Wycombe, United Kingdom) or by commercial providers (J. Bartley, Natural History Museum, London, United Kingdom; MWG Biotech, Milton Keynes, United Kingdom; Cytomix, Cambridge, United Kingdom).

Primers. Initially, previously described primers were used to amplify a small region of the *pol* gene (9, 28). PolOR was used for cDNA synthesis and first-round PCR with Polis4; Polis4/Unipol2 were then used for second-round PCR. New primers were designed in conserved regions of the *gag* (MN1, MN2, and MN3), *env* (MN4, MN5, and MN6) and *nef* (MN11 and MN14) genes based on multiple alignments of previously published SIV and HIV sequences. The sequences of these primers and their locations relative to SIVrcmNG411 (accession no. AF349680) are as follows (Fig. 2): MN1 (GARGAAGSDGCMASANTGGGA; 826 to 847), MN2 (ATNCARAATGCHAAAYCCDGAHTG; 1184 to 1206), MN3 (GCHTGYCARGGVGTHGGDGG; 1262 to 1281), MN4 (TKGAGRTTYTNRYWCCCA; 7666 to 7685), MN5 (CARVTKYTKCTGYTGCTGCAVTATCCC; 7594 to 7620), MN6 (CCCATTGCAGHDCCHGC; 7531 to 7547), MN11 (CCTTCAGTCCHCCCTTBHTTTTA; 8683 to 8709), and MN14 (DBNGRDTYVAABDDCCA; 8954 to 8970).

In addition to the predicted site in *env*, MN6 also primed between *pol* and *env*. In subsequent work, MN14, MN4, and PolOR were used for cDNA synthesis and then as first-round PCR primers together with MN1, Polis4, or sequence-specific primers designed in the small *pol* region between Polis4 and Unipol2. Second-round PCR primers included MN2, MN3, MN5, MN6, MN11, Unipol2, and sequence-specific primers designed from sequence obtained from the PCR walk. These included the following: MN7 (CATTGAACCAGCCCGAGA), MN8 (TCTAGCCAAAGAGATAGTCA), MN10 (CGGCTACTAGGATGATACTTC), MN28 (CTACAGCAGCCCAAAGGAGA), and MN30 (GATAATCCGGAGTCAAGT).

Sequence and phylogenetic analysis. Sequences were analyzed by using programs in the Lasergene suite (DNASTar, Madison, Wis.). CLUSTAL alignments (47) were edited by eye in MacClade 4.0.2 (D. R. Maddison and W. P. Maddison, Sinauer Associates, Sunderland, Mass.), exported into PAUP* 4.0.1 (D. L. Swofford, Sinauer Associates) for gap stripping and first- and second-codon position alignments. Concatenated *gag*, *pol*, *vif*, *env*, and *nef* nucleotide and corresponding amino acid alignments were also made. Phylogenetic reconstructions were carried out by using PAUP*, and the most appropriate evolutionary models and parameters were chosen by using Modeltest (35). Tree searches were carried out by using a heuristic search strategy and branch swapping. Neighbor

joining-derived trees were bootstrapped 1,000 times. Protdist (J. Felsenstein, Department of Genetics, University of Washington, Seattle [http://evolution.genetics.washington.edu/phylip/software.html]) was used to estimate the amino acid similarity of aligned predicted protein sequences. Trees were displayed with Treeview (31), RIP (42) and SimPlot (S. Ray, Division of Infectious Diseases, Department of Medicine, Johns Hopkins University School of Medicine [http://sray.med.som.jhmi.edu/RaySoft/SimPlot/]) were used for similarity and boot-scanning analyses (26). Oligo (MedProbe, Oslo, Norway) was used to design SIVmonNG1 sequence-specific primers.

Nucleotide sequence accession numbers. Nucleotide sequences have been submitted to the EMBL database under accession numbers AJ549283 and AJ549756.

RESULTS

Identification of HIV antibody-positive serum. Serum samples were obtained from two mona monkeys at Cercopan in Nigeria. One was found to be reactive in both EIA tests, while the other was unreactive. In the Western blot, the EIA-positive sample was strongly reactive with the HIV-1 p66 antigen (the RT) and had weaker reactivities with gp160 (envelope), p31 (the integrase), and p24 (core) antigens of HIV-1, as well as the HIV-2 p36 transmembrane antigen (Fig. 3). This indicated that the mona monkey was infected with a primate lentivirus.

Amplification of SIVmonNG1 RNA. RNA was extracted from the serum and subjected to nested reverse transcription-PCR with conserved primers in the integrase gene: Polis4/PolOR and then Polis4/Unipol2 (9, 28). The 598 nucleotides of sequence that were derived from this amplicon were found to be phylogenetically related to other primate lentivirus genome sequences, thus confirming that the mona monkey was infected with a novel SIV. Degenerate primers were then designed based on multiple alignments of either *gag*, *env*, or *nef* SIV and HIV sequences in order to be able to extend beyond the *pol* sequences obtained by using Polis4/Unipol2. These primers (MN1, MN2, and MN3 in *gag*; MN4, MN5, and MN6 in *env*; MN11 and MN14 in *nef*) were used in RT nested-PCR protocols (Fig. 2). For example, MN4 was used as an RT primer and

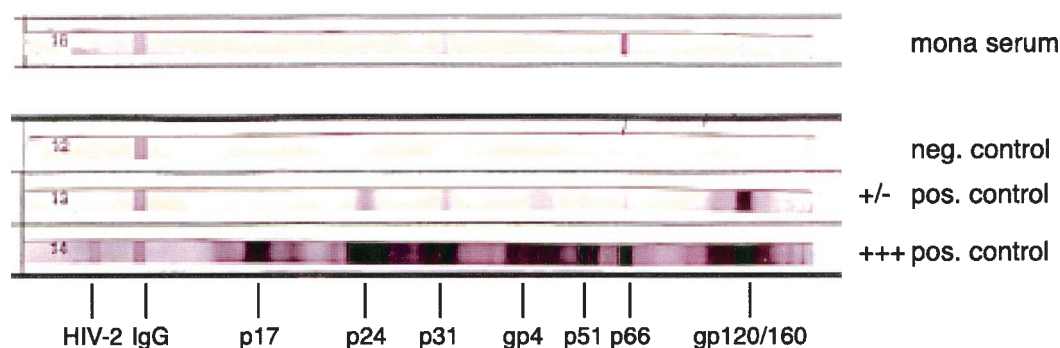


FIG. 3. Antibody profile of serum from the mona monkey revealed on a Genelabs Western blot. At the top is the serum from the mona monkey; below are a negative human serum control, a weak anti-HIV-1-positive human serum control, and a strong anti-HIV-1-positive human serum control.

then MN1 and MN4 or MN5 as first-round primers. Alternatively, PolOR or MN14 were used to prime cDNA synthesis. Various combinations of second-round primers were then used, including MN2/Unipol2 and MN1, MN6, and MN11 with specific primers designed on the basis of sequence information derived from amplicons produced with Polis4/Unipol2 (MN7, MN8, and MN10) or with MN7/MN6 (MN28 and MN30). The PCR enzymes and conditions were critical to the successful use of these primers; we mostly used an enzyme mixture with a proofreading polymerase, together with a low annealing temperature for the first 10 cycles of the PCR. By these methods we were able to amplify and clone regions of the genome from about two-thirds into the *gag* gene to the 3' poly(A) tail (Fig. 2).

Genomic organization of SIVmonNG1: presence of a *vpu* gene. Open reading frames (ORFs) in the sequences were identified by BLAST searches of the Los Alamos HIV sequence database (<http://hiv-web.lanl.gov>) and by nucleotide and derived amino acid phylogenetic comparisons with other SIV sequences. Potential Gag, Pol, Vif, Tat, Vpr, Rev, and Nef proteins were identified. In addition, an ORF in a similar position to the *vpu* gene of HIV-1 and SIVcpz was identified (Fig. 2). Therefore, SIVmonNG1 has a genomic organization more similar to HIV-1, SIVcpz, and SIVgsn than to the HIV-2/SIVsm lineage viruses or to the SIVagm, SIVlhoest, SIVsyk, or SIVcol lineages (5).

Putative Vpu amino acid sequence. The SIVmonNG1 putative Vpu amino acid sequence was not closely related to other Vpu sequences, either HIV-1, SIVcpz, or SIVgsn. The amino acid similarity of SIVmonNG1 Vpu to other Vpu proteins was estimated by using the PHYLIP program Protdist with a manually adjusted CLUSTAL W amino acid alignment and ranged from 15.2% for SIVcpzUS to 17.9% for HIV-1 to 23.4% for SIVgsn to 29% for SIVcpzANT (Table 1). Like all Vpu proteins, the N-terminal half of the sequence (residues 6 to 33) has many hydrophobic residues and is characteristic of a transmembrane domain. The C-terminal half of the sequence has many acidic residues but not the EDSGNESXG(E/D) domain that is highly conserved among HIV-1 and SIVcpz strains, nor does it have a similar sequence to SIVgsn in this region (10). It does, however, have the run of glutamic and aspartic acid residues present in all Vpu proteins, and it also has a DNP

motif near the C terminus, in common with other HIV-1 and SIVcpz Vpu sequences.

Genomic relatedness of SIVmonNG1 to other SIVs. The relationship of SIVmonNG1 to other primate lentiviruses was investigated by comparing the sequence obtained to representatives of the six other SIV lineages and to SIVgsn. First, diversity plots were performed by using a multiple alignment of concatenated predicted Gag, Pol, Vif, Env, and Nef gene products. Ambiguous sites, as well as sites that contained a gap, were removed from the alignment. The sequence similarity was calculated for a window of 200 amino acids moved along the alignment (Fig. 4). The SIVmonNG1 sequence showed the greatest similarity to SIVgsn across the whole genome, and the most diverse protein was Vif.

The second method used for examining the relationship of SIVmonNG1 to other SIV genomes was bootscanning (Fig. 5). For this, as for subsequent phylogenetic analyses, third-codon bases were removed from the alignment since they are saturated with nucleotide substitutions and have been shown to be phylogenetically uninformative (10). Bootscanning against single sequences from the six major SIV lineages showed that the SIVmonNG1 genome is a complex mosaic, with the 5' region related to SIVsyk and SIVcol and the 3' region related to SIVcpz and SIVsyk sequences (Fig. 5A). When SIVgsn and SIVcpzANT sequences were included in the bootscanning analysis, the SIVmonNG1 sequence was seen to be most closely related to SIVgsn, apart from a small region in *pol*

TABLE 1. Amino acid sequence identity between SIVmonNG1 and other SIV and HIV-1 genomes in Vpu

Sequence	Amino acid similarity (%)						
	gsn71	cpzUS	cpzANT	cpzGAB	M	N	O
SIVmonNG1	23.4	15.2	29.0	20.9	17.9	18.5	23.9
SIVgsn71	100	20.3	23.9	16.9	15.9	20.0	21.7
SIVcpzUS		100	24.6	32.9	35.6	44.3	30.4
SIVcpzANT			100	24.6	20.0	29.2	28.2
SIVcpzGAB				100	32.9	28.2	31.3
HIV-1 M Z2Z6					100	42.9	28.4
HIV-1 N YBF						100	27.6
HIV-1 O ANT70							100

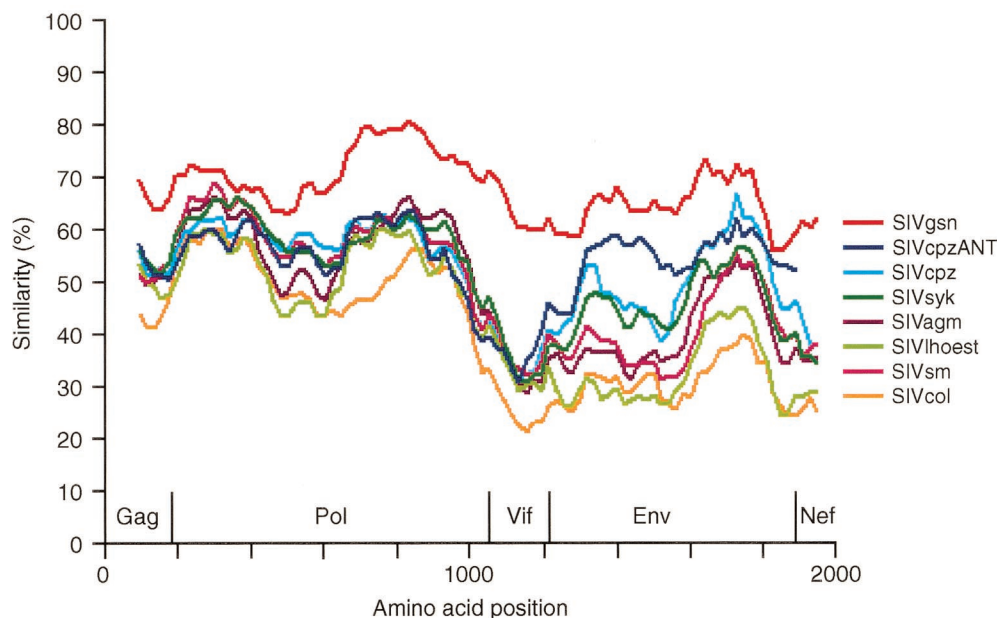


FIG. 4. Similarity plotting of SIVmonNG1 against the six major SIV lineages (SIVsyk, SIVlhoest, SIVcol, SIVagm, SIVcpz, and SIVsm) and SIVcpzANT and SIVgsn. Alignments of the nonoverlapping regions of *gag*, *pol*, *vif*, *env*, and *nef* were concatenated together; gaps were removed from the amino acid alignment; a window of 200 residues was used. The relative position of the genome proteins is shown. Sequences for comparison were obtained from the Los Alamos HIV Database/GenBank.

resembling SIVsyk (Fig. 5B). This substantiates the conclusion that SIVmonNG1 and SIVgsn form a shared lineage.

Comparing the predicted protein sequences of SIVmonNG1 with representatives of other primate lentivirus lineages, SIVmonNG1 was most closely related to SIVgsn (Table 2). For example, for *Pol*, the similarity score ranged from 34.9% with SIVcpzGAB to 68.3% with SIVgsn. For *Env*, the similarity ranged from 30.1% with SIVcol to 56.2% for SIVcpzANT to 61.9% for SIVgsn. The SIVmonNG1 *Env* protein V3 loop is predicted to be 35 amino acids in length and has a QIGAGM TFYS central motif identical to that of SIVgsn; overall, these two V3 loops have 94% identity. The SIVmonNG1 V3 loop is also closely related to SIVcpzANT (71% identity) and similar to other SIVcpz V3 sequences.

The predicted mosaicism of the genome of SIVmonNG1 observed by bootscanning was further analyzed by phylogenetic analysis of selected genomic regions. Several gene regions were investigated, but only the *env* and *pol* gave trees with significant bootstrap support for the branching of the SIVmonNG1 and SIVgsn sequences. Analysis of the *env* gene by both standard alignments and gap-stripped alignments, and by first- and second-codon positions only, all gave rise to phylogenetic trees in which SIVmonNG1 *env* clustered with SIVgsn sequences. For example, Fig. 6 shows a phylogenetic tree derived by maximum likelihood searching, in which the best-fitting model of evolution was evaluated by using Modeltest and PAUP* (35). Support for the branching order was found by bootstrapping (1,000 times) a congruent neighbor-joining tree derived with the same model of evolution. Further, the SIVmon and SIVgsn *env* sequences also formed a clade with HIV-1/SIVcpz *env* sequences, supported by a 94 bootstrap proportion, further strengthening the idea that these envelope genes are related.

In contrast to the *env* gene, when the *pol* gene was examined by similar phylogenetic methods the SIVmonNG1 sequence formed a clade with SIVgsn and SIVsyk, separate from SIVcpz/HIV-1 sequences (Fig. 7). Using bootstrapping with distance methods a high (98 bootstrap proportion) support was found for this grouping. Recently, partial *pol* sequences (522 nucleotides) have been determined for several previously uncharacterized SIVs from African primates in Cameroon (33), including mona monkeys and the mustached monkey (*Cercopithecus cephus*). Comparing the equivalent region from SIVmonNG1 indicated that it was closest to another mona monkey sequence, SIVmon-99CM-CML1 (Fig. 8). These sequences formed a separate cluster with the *Cercopithecus nictans* (SIVgsn-99CM-CN71) and *Cercopithecus cephus* (SIVmus-01CM-S1239) sequences, away from other SIV sequences, including SIVsyk, which grouped with SIVtal (Fig. 8). Additional sequence data from SIVmus and SIVtal, and any as-yet-uncharacterized SIVs, will enable greater understanding of the relationships between these SIVs.

DISCUSSION

The sequence of more than 8,200 bases of the RNA genome [partial *gag* to 3' poly(A)] of an SIV from a mona monkey (*Cercopithecus mona*) was derived by a PCR amplification strategy by using novel degenerate primers located in conserved regions of SIV/HIV genomes. Attempts to recover the 5' end by RACE and amplification with a primer based on the presumptive tRNA-binding site were unsuccessful, and insufficient sample remained for further work. Further characterization of the SIVmonNG1 genome will, therefore, require the collection of more serum. The SIVmonNG1 sequence was obtained from serum collected from a young wild-born mona

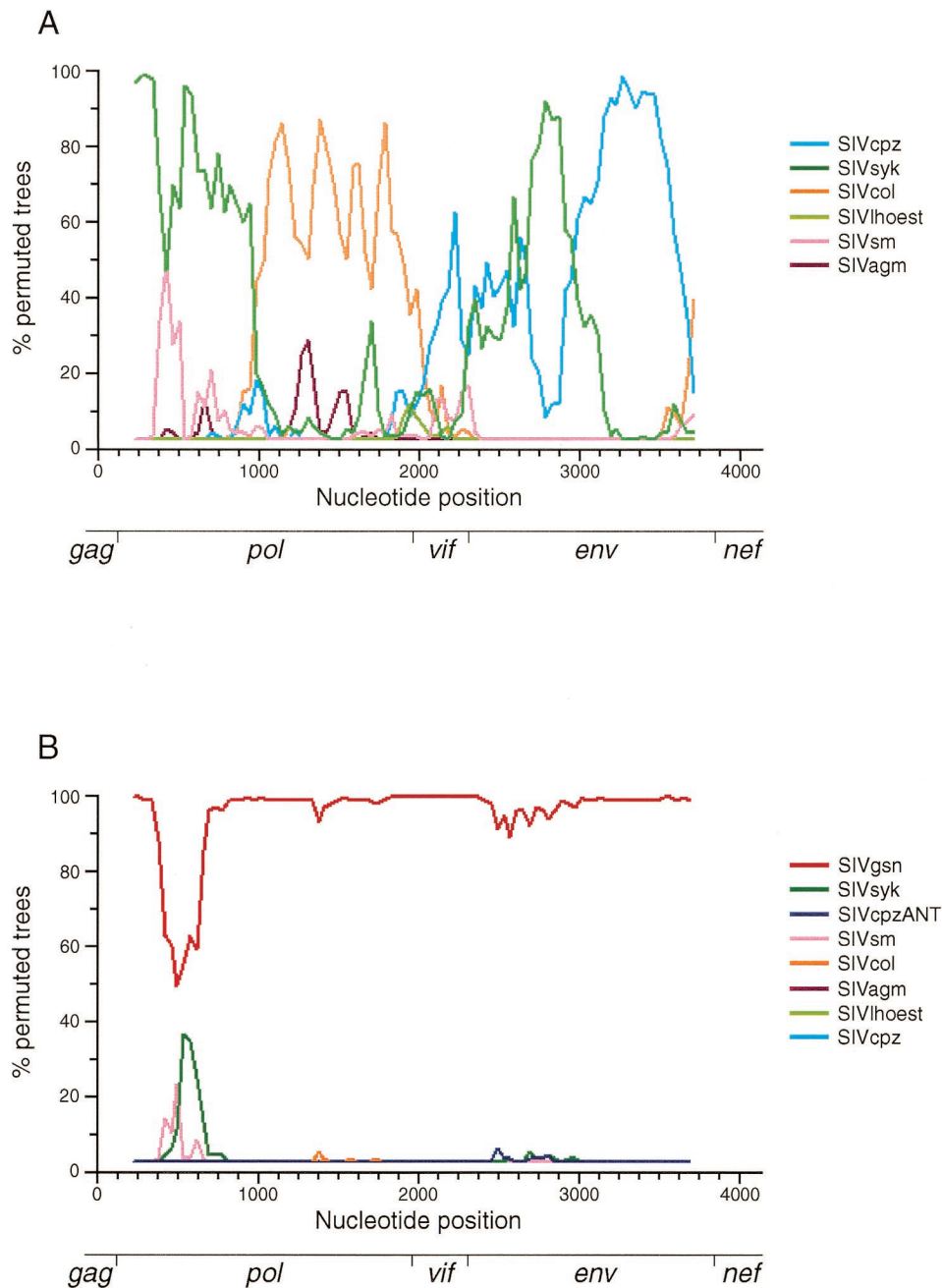


FIG. 5. (A) DNA bootscanning comparisons of SIVmonNG1 against single sequences from the major SIV lineages (SIVsyk, SIVlhoest, SIVcol, SIVagmGRI, SIVcpz, and SIVsm). (B) Similar analysis including SIVgsn and SIVcpzANT. Alignments of the nonoverlapping regions of *gag*, *pol*, *vif*, *env*, and *nef* were concatenated together, and third-codon positions and gaps were excluded. A sliding window of 450 bases in 40 base steps and 1,000 bootstrap replicates was used for bootscanning with the Kimura 2 parameter model and a transition/transversion ratio of 0.75. The relative positions of the genes are shown at the bottom of the figures.

monkey in the Cercopan Conservation Centre in Nigeria. As with any primate lentivirus that can jump from one species to another, it has to be asked whether the mona monkey is the primary host for this virus or whether it could have come from another monkey. The young age of the monkey (<2 months old) is consistent with it being the primary host. The availability of a small *pol* region sequence independently obtained from a mona monkey from the Cameroon (33) allowed a comparison with the sequence we obtained (Fig. 8). The two sequences

were clearly related (82% identity over 522 nucleotides), a finding consistent with them being the same SIV from animals at extremes of the species range, which is from Ghana to the Cameroon (Fig. 1) (36). We do not have any evidence that SIVmonNG1 was associated with any specific disease.

The molecular characterization of this genome provides new insights into the phylogeny and evolution of the primate lentiviruses. Significantly, the genome has a putative *vpu* gene, which has previously only been found in HIV-1 and SIVcpz

TABLE 2. Amino acid sequence identity between SIVmonNG1 and other SIV isolates

Sequence	Amino acid similarity (%) ^a								
	Gag	Pol	Vif	Vpr	Tat	Rev	Vpu	Env	Nef
SIVgsn71	65.4	68.3	64.0	67.7	46.8	19.4	23.4	61.9	71.4
SIVcpzANT	52.1	36.2	29.9	28.6	53.0	18.9	29.0	56.2	NA
SIVcpzGAB	53.6	34.9	26.2	27.8	46.3	19.1	20.9	49.9	33.7
SIVsyk	53.3	48.9	29.4	24.2	42.4	17.6	NA	45.7	32.2
SIVsm	45.0	37.1	28.1	26.1	30.0	20.2	NA	40.5	39.4
SIVagmGRI	45.3	40.9	23.7	28.9	36.8	21.2	NA	39.8	36.7
SIVlhoest	44.8	54.6	24.0	19.8	29.4	9.4	NA	31.8	33.8
SIVcol	39.9	48.5	26.4	24.4	38.9	17.2	NA	30.1	24.7

^a NA, not applicable or available. Values in boldface indicate highest sequence identity scores.

genomes and, more recently, in SIVgsn (10). The conceptual translation of this gene yields a sequence that is consistent with it being related to the Vpu proteins of HIV-1, SIVcpz, and SIVgsn although, like these proteins, it is clearly divergent with

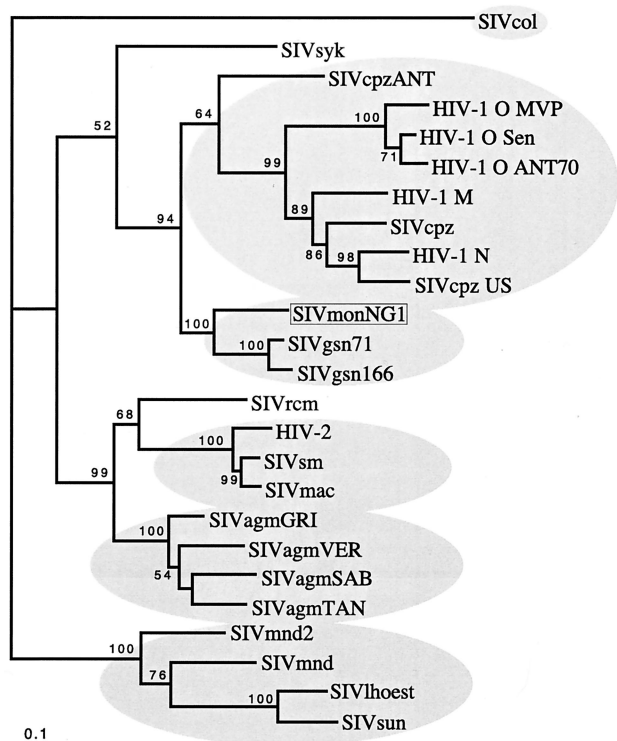


FIG. 6. Phylogenetic comparison of *env* SIV and HIV sequences. An in-frame nucleotide alignment of *env* sequences was derived from the corresponding amino acid alignment, and gaps and codon third-position bases were excluded. The best-fitting model of evolution found by using Modeltest (35) with PAUP* was the general time reversible with the following parameters. The rates were as follows: A-C = 1.3738, A-G = 0.9821, A-T = 0.3369, C-G = 1.5349, C-T = 1.2473, and G-T = 1.0. The base frequencies were as follows: A = 0.3135, C = 0.1323, G = 0.2273, and T = 0.3269. The gamma distribution shape parameter (alpha) was 4.6652, and the proportion of invariable sites was 0.0093. The branch lengths of the tree shown were derived by a maximum-likelihood search with the above parameters. The bootstrap values (of 1,000) were derived from a congruent distance/neighbor-joining tree found with the same model of evolution. The scale bar indicates the number of nucleotide substitutions per site. Sequences for comparison were obtained from the Los Alamos HIV Database/GenBank.

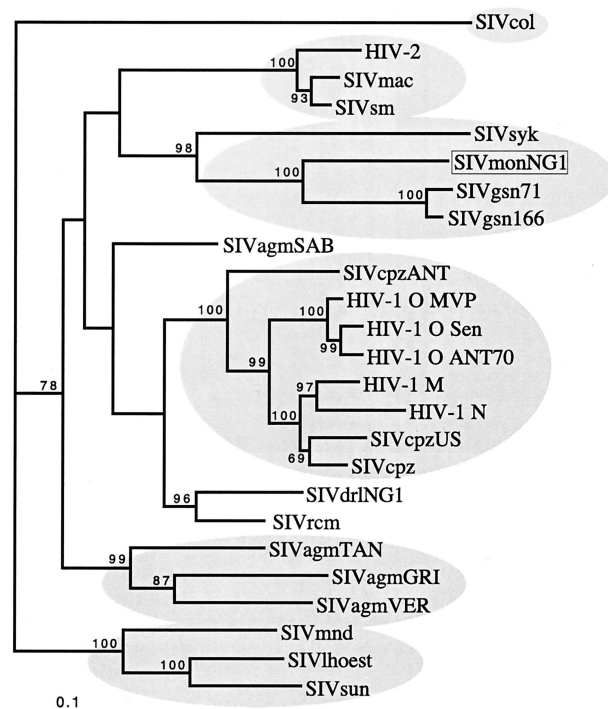


FIG. 7. Phylogenetic comparison of *pol* SIV and HIV sequences. An in-frame nucleotide alignment of *pol* sequences was derived from the corresponding amino acid alignment, and gaps and codon third-position bases were excluded. The best-fitting model of evolution found by using Modeltest (35) with PAUP* was the general time reversible. The rates were as follows: A-C = 2.9306, A-G = 3.2906, A-T = 1.9048, C-G = 3.1502, C-T = 5.2621, and G-T = 1.0. The base frequencies were as follows: A = 0.3709, C = 0.2021, G = 0.2353, and T = 0.1917. The gamma distribution shape parameter was alpha = 1.2972. The proportion of invariable sites was 0.2594. The branch lengths of the tree shown were derived by a maximum-likelihood search with the above parameters. The bootstrap values (of 1,000) were derived from a congruent distance/neighbor-joining tree found with the same model of evolution. The scale bar indicates the number of nucleotide substitutions per site. Apart from SIVmonNG1, sequences were obtained from the Los Alamos HIV Database/GenBank.

an average similarity of 21% (Table 1). HIV-1 Vpu has two domains: an N-terminal transmembrane anchor and a C-terminal cytoplasmic domain (38). The transmembrane domain enhances virus release from infected cells, whereas the cytoplasmic domain is involved in the degradation of CD4 (32, 37, 48). When the derived protein sequence of SIVmonNG1 Vpu was analyzed by molecular modeling (data not shown), the amino terminus (residues 6 to 33) was found, as expected, to be characteristic of a transmembrane domain. The C-terminal (cytoplasmic) half, however, was predicted to have a single alpha-helix, located before two candidate phosphoserine residues. The SIVgsn Vpu was also predicted to have only one alpha-helix. In comparison, by using the same prediction algorithms, HIV-1 and SIVcpz Vpu proteins had two alpha-helices, one on either side of the phosphoserines, a finding consistent with the structural data (12, 52, 53). This could be interpreted as indicating a difference in biological activity of the cytoplasmic domain of the SIVmonNG1 Vpu compared to the equiv-

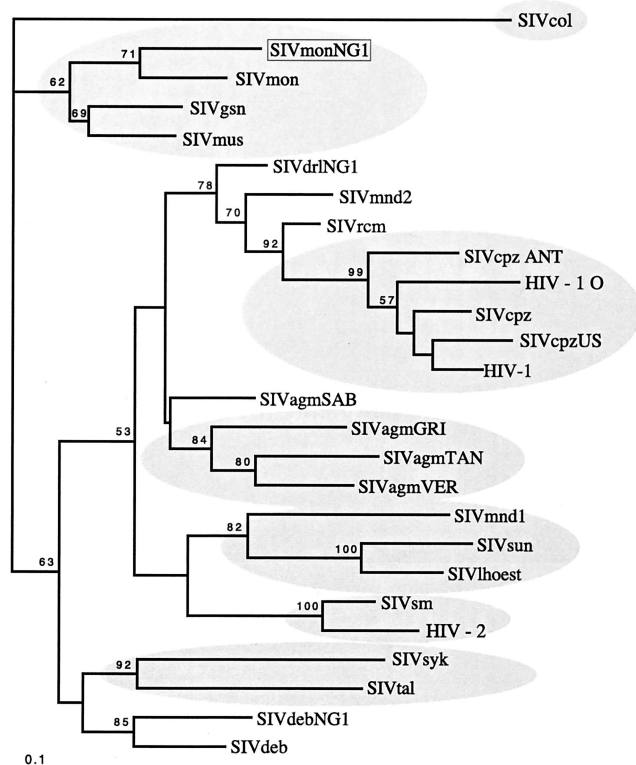


FIG. 8. Maximum-likelihood tree of a 520-bp *pol* sequence, with significant bootstrap values (of 100) indicated. The sequences represent the amplicon derived by using the Polis4/Unipol2 primers (9, 28). The best-fitting model of evolution found by using Modeltest (35) with PAUP* was the general time reversible. The rates were as follows: A-C = 2.4379, A-G = 5.8145, A-T = 2.2893, C-G = 3.5281, C-T = 12.703, and G-T = 1.0. The base frequencies were as follows: A = 0.4341, C = 0.1827, G = 0.2021, and T = 0.1811. The gamma distribution shape parameter was $\alpha = 0.9683$. The proportion of invariable sites was 0.2127. Both the branch lengths and the bootstrap proportions (of 100) were derived by maximum-likelihood searching. The scale bar indicates the number of nucleotide substitutions per site. SIVmonNG1 (AJ549283), SIVdrING1 (AJ310481), and SIVdebNG1 (AJ549756) were sequenced as part of the present study; SIVmon (99CM-CML1), SIVgsn (99CM-CN71), SIVmus (01CM-S1239), SIVmnd2, SIVtal, and SIVdeb are from Peeters et al. (33); these latter sequences and others were obtained from the Los Alamos HIV Database/GenBank.

alent region of the HIV-1/SIVcpz protein. Of course, this would need to be established experimentally.

Sequence comparisons of the SIVmonNG1 genome further illustrate the complexity of the relationships between the primate lentiviruses. The SIVmonNG1 sequence is most closely related to SIVgsn, as can be seen from the amino acid similarity plot (Fig. 4) and, in common with that genome, it can be interpreted as having a complex mosaic structure, as shown by bootscanning (Fig. 5A). Aside from SIVgsn, the 5' half of the SIVmonNG1 genome shows similarity to SIVsyk and SIVcol by bootscanning. Again, apart from SIVgsn (Fig. 5B), the 3' half of the genome resembles SIVcpzANT by similarity plotting and SIVsyk and SIVcpz by bootscanning. The *vif* gene is the most diverse region of the SIVmonNG1 genome (Fig. 4).

More definitive analyses of the genetic relationships of SIVmonNG1 were attempted by phylogenetic comparisons of selected genome regions. Trees derived from alignments of

small genome regions suggested by the bootscans have did not high bootstrap support, as was also observed for SIVgsn (10). Trees made from the *pol* and *env* regions were, however, unambiguous. The *env* region of SIVmonNG1 and SIVgsn formed a clade with SIVcpz/HIV-1 with high bootstrap support (94 BP) (Fig. 6). It is perhaps not surprising that *env* sequences resembling those of the HIV-1/SIVcpz clade are found in another SIV that has a *vpu* gene, since the *env* and *vpu* genes overlap and are translated from a bicistronic mRNA (39). Thus, the *env* gene similarity and the presence of a *vpu* gene could be interpreted as indicating that the *vpu/env* gene regions of SIVmonNG1, SIVgsn, and SIVcpz have a common origin or ancestor. Analysis of the *pol* region showed that SIVmonNG1, SIVsyk, and SIVgsn formed a clade with high bootstrap support (98 BP) (Fig. 7), indicating that the *pol* gene region of SIVmonNG1 has a different origin from the *env* gene region. Thus, from the similarity plots, bootscans, and phylogenetic trees, it is clear that SIVmonNG1, like SIVgsn, has a complex mosaic genome, related to the SIVs of other *Cercopithecinae* monkeys. The present-day ranges of the mona monkey and those of the four other species (chimpanzee, greater spotted monkey, Sykes monkey, and colobus monkey), whose SIVs show the most similarity to SIVmonNG1, are shown in Fig. 1. The ranges of the mona monkey, chimpanzee, greater spot-nosed monkey, and colobus monkey overlap. The Sykes monkey range is adjacent to that of the greater spot-nosed monkey and overlaps with the colobus monkey. This geographical distribution, as well as the similarity of the SIVmonNG1 and SIVgsn genomes from distinct species, is consistent with the idea that the same lineage has infected both species or that SIVgsn passed from the greater spot-nosed to the mona monkey.

The similarity of SIVmonNG1 and SIVgsn to SIVcpz in *env* raises the question of whether they or the SIVcpz lineage are recombinant. Previously characterized recombinants include SIVagmSAB from the sabaeus African green monkey (23), SIVrcm from red-capped mangabeys (5, 15), SIVmnd-2 from the mandrill (43), and SIVdrl from the drill monkey (8) (Clewley, unpublished). As Souquière et al. have pointed out, the *Cercopithecinae* monkeys, the mandrills, and drill monkeys are close relatives (43), and the existence of recombinant forms in these species supports the suggestion that the SIVs from them are ancestral and that recombinants play a major role in primate lentivirus evolution (3, 17, 25). The present study provides evidence consistent with an SIVcpz ancestral or progenitor virus being present in a *Cercopithecinae* monkey, suggesting that the SIVcpz group is mosaic. As more SIV genomes are characterized, it is likely to become increasingly difficult to recognize pure lineages.

The PCR primers we used to recover sequence information from SIVmonNG1 RNA in serum may help the characterization of previously unknown SIVs. Many recent SIV studies have used proviral DNA from cultured infected cells as the starting point for the recovery of whole-genome sequence information, either by PCR amplification of unintegrated circular DNA or by screening a genomic library (2, 3, 5, 9, 10, 15, 17–19, 43). These experiments have often relied on degenerate primers to obtain starting sequence information or for inverse PCR of circular DNA (5, 9, 10, 15, 17, 43). Several sets of degenerate primers have been designed and used by different

investigators to obtain, for example, small regions of sequence information in the conserved *pol* gene (8, 9, 14, 28). Perhaps the most comprehensive use of degenerate primers has been Peeters et al. study of SIV sequences recovered from bushmeat in Cameroon (33).

In conclusion, we have identified a novel SIV RNA sequence from *Cercopithecus mona* that has a mosaic (recombinant) genome and which has a *vpu*-like gene, potentially expressing a Vpu protein. Collection of further samples will be necessary for characterization of the 5' end of the genome and growth of the virus.

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