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Characterization of Novel Simian Immunodeficiency Viruses from Red-Capped Mangabeys from Nigeria (SIVrcmNG409 and -NG411)

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Two novel simian immunodeficiency virus (SIV) strains from wild-caught red-capped mangabeys (*Cercocebus torquatus torquatus*) from Nigeria were characterized. Sequence analysis of the fully sequenced SIV strain rcmNG411 (SIVrcmNG411) and *gag* and *pol* sequence of SIVrcmNG409 revealed that they were genetically most closely related to the recently characterized SIVrcm from Gabon (SIVrcmGB1). Thus, red-capped mangabeys from distant geographic locations harbor a common lineage of SIV. SIVrcmNG411 carried a *vpx* gene in addition to *vpr*, suggesting a common evolutionary ancestor with SIVsm (from sooty mangabeys). However, SIVrcm was only marginally closer to SIVsm in that region than to any of the other lentiviruses. SIVrcm showed the highest similarity in *pol* with SIVdrl, isolated from a drill, a primate that is phylogenetically distinct from mangabey monkeys, and clustered with other primate lentiviruses (primarily SIVcpz [from chimpanzees] and SIVagmSab [from African green monkeys]) discordantly in different regions of the genome, suggesting a history of recombination. Despite the genetic relationship to SIVcpz in the *pol* gene, SIVrcmNG411 did not replicate in chimpanzee peripheral blood mononuclear cells (PBMC), although two other viruses unrelated to SIVcpz, SIVmndGB1 (from mandrills) and SIVlhoest (from L'Hoest monkeys), were able to grow in chimpanzee PBMC. The CCR5 24-bp deletion previously described in red-capped mangabeys from Gabon was also observed in Nigerian red-capped mangabeys, and SIVrcmNG411, like SIVrcmGB1, used CCR2B and STRL33 as coreceptors for virus entry. SIVrcm, SIVsm, SIVmndGB1, and all four SIVlhoest isolates but not SIVsun (from sun-tailed monkeys) replicated efficiently in human PBMC, suggesting that the ability to infect the human host can vary within one lineage.

Simian immunodeficiency viruses (SIVs), which together with the human immunodeficiency viruses type 1 and type 2 (HIV-1 and HIV-2) constitute the primate lentivirus family, are harbored naturally in African primates. Currently, SIV infection has been detected in more than 20 different species of African primates (4, 7, 28, 34, 35). The primate lentiviruses can currently be classified into six distinct lineages based upon phylogenetic relationships (5). The six known primate lineages are approximately equidistant from one another, differing by approximately 40 to 50% in the Pol protein. These lineages are represented by (i) SIVcpz from chimpanzees (*Pan troglodytes*) including HIV-1 (13, 21, 22, 38, 46), (ii) SIVsm from sooty mangabeys (*Cercocebus atys*) including HIV-2 and SIVmac from macaques (*Macaca* spp.) (17, 20, 33), (iii) SIVagm from African green monkeys (members of the *Chlorocebus aethiops* superspecies) (1, 2, 9–11, 19, 23, 24, 32), (iv) SIVsyk from Sykes' monkeys (*Cercopithecus albogularis*), (v) SIVlhoest from L'Hoest monkeys (*Cercopithecus l'hoesti*) including SIVsun from sun-tailed monkeys (*Cercopithecus solatus*) and SIVmnd from mandrills (*Mandrillus sphinx*) (3, 4, 18, 45), and most recently discovered (vi) SIVcol from Colobus monkeys (*Colobus guereza*) (8). Some SIVs, such as SIVdrl from drills (*Mandrillus leucophaeus* [7]), SIVrcm from red-capped mangabeys (*Cercocebus torquatus torquatus* [14]) and SIVtal from talapoin monkeys (*Miopithecus talapoin* [35]), have only been partially characterized; sequence analysis of the entire genomes of these viruses will be required for definitive classification.

The majority of SIV strains described to date can be clearly classified into one of these six primate lentivirus lineages. However, there are instances in which the phylogenetic position of SIV strains varies depending upon the regions of the genome analyzed, implicating recombination events in the past. SIV from the African green monkey from West Africa (*Chlorocebus sabaeus*), SIVagmSab, represents an example of such a mosaic genome structure (23). Most of the SIVagmSab genome clusters with the other members of the SIVagm lineage (SIVagmVer, SIVagmGri, and SIVagmTan, from vervet, grivet, and tantalus subspecies, respectively); however, the 3′ portion of *gag* and the 5′ portion of *pol* of SIVagmSab were highly divergent from the other SIVagm subtypes and appeared to cluster with the viruses of the SIVsm/HIV-2 lineage (23) (see Fig. 5A). Partial characterization of the *gag* (954 nt) and *pol* (475 nt) genes of SIVrcmGB1 from a red-capped

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mangabey from Gabon suggested that this virus may also represent a recombinant between different primate lentivirus lineages (14). SIVrcm formed a novel lineage in *gag* but clustered with the SIVcpz/HIV-1 lineage in *pol* (14), suggesting a mosaic or recombinant structure. Clewley et al. (7) demonstrated that a partial sequence of the *pol* gene (787 nt) of SIVdrl from a drill monkey showed the closest phylogenetic relationship to SIVcpz and SIVagmSab; however, the relationship of SIVdrl and SIVrcm could not be determined since separate regions of *pol* were analyzed for these two viruses.

In the present report, we identified two additional SIV-seropositive red-capped mangabeys in Nigeria, the northern extreme of the range of this species. SIV was isolated from one of the seropositive mangabeys (SIVrcmNG411) and characterized for tropism and coreceptor usage. Coreceptor usage of SIVrcmNG411 was of considerable interest since the previously reported SIVrcmGB1 strain was found to use CCR2B as primary coreceptor for virus entry, rather than CCR5, the major SIV coreceptor (6). The lack of CCR5 use by SIVrcm may be due to the high prevalence of a CCR5 allele with a 24-bp in-frame deletion among red-capped mangabeys. This CCR5 allele was observed with high frequency in red-capped mangabeys from both Gabon and North American zoos, suggesting that this is a common feature of this species (6). Thus, an additional goal of the present study was to analyze CCR5 gene allele frequencies in Nigerian red-capped mangabeys. Finally, the entire genome of SIVrcmNG411 and the *gag* and *pol* genes of SIVrcmNG409 were molecularly characterized.

MATERIALS AND METHODS

Animals and samples. Blood samples were collected from 13 red-capped mangabeys, captured as orphans and housed in a monkey sanctuary in southeast Nigeria (near the Cameroon border). The plasma was separated by low-speed centrifugation shortly after sampling, and peripheral blood mononuclear cells (PBMC) were separated by Ficoll-sodium diatrizoate (LSM; ICN Biomedicals, Aurora, Ohio) density gradient centrifugation of whole blood. Both plasma and PBMC samples were stored in liquid nitrogen until use.

SIV serology. Serology for antibodies to SIV was performed by radioimmunoprecipitation assay as described previously (4). Briefly, CEMx174 cells were infected with SIVsmE660 (15) and at the peak of reverse transcriptase (RT) activity, labeled overnight with L-[^{35}S]methionine and L-[^{35}S]cysteine (Amersham, Arlington Heights, Ill.). The labeled cells were lysed the next day and centrifuged, and the cell lysate supernatant was preabsorbed with 50 μl of protein A-agarose beads (Gibco BRL, Gaithersburg, Md.) for 1 h. Ten microliters of monkey plasma was combined with 50 μl of protein A-agarose beads and incubated with shaking for 1 h at 4°C. The protein A-agarose bead-antibody complex was combined with equal aliquots of cell lysate, incubated with shaking for 1 h at 4°C, and then washed five times with a detergent buffer. The pellet was resuspended in sodium dodecyl sulfate (SDS) gel loading buffer (100 mM Tris-HCl [pH 6.8], 200 mM 2-mercaptoethanol, 4% SDS, 0.2% bromophenol blue, 20% glycerol), boiled for 4 min, and then loaded onto a 10% SDS polyacrylamide gel. The dried gel was exposed to a Kodak Bio-Max MR film (Kodak, Rochester, N.Y.) for 5 days.

Virus isolation. PBMC (1×10^7 to 2×10^7) of the two SIV-positive red-capped mangabeys (RCM409 and RCM411) were stimulated with 2 μg of phytohemagglutinin (PHA; Sigma, St. Louis, Mo.) per ml for 4 days and then depleted of $CD8^+$ cells using Dynabeads (Dynal, Oslo, Norway). The $CD8^-$ fraction of PBMC was incubated with PHA-stimulated human PBMC in complete RPMI with 10% fetal calf serum (FCS), supplemented with 5 half-maximal units of human interleukin-2 (Advanced Biotechnologies, Columbia, Md.) per ml. Culture supernatants were collected every 3 days and subjected to a ^{32}P-based RT assay (37).

PCR amplification and plasmid cloning. PCRs were performed using 0.5 μg of genomic DNA. For amplification of SIVrcmNG411, DNA was extracted from PHA-stimulated SIV-producing red-capped mangabey PBMC. Diagnostic *pol* primers were used from a highly conserved area of the *pol* gene (30); these were UNIPOL1 (5′-AGTGGATTCATAGAAGCAGAAGT-3′) and UNIPOL2 (5′-CCCCTATTCCTCCCCTTCTTTTAAAA-3′). PCR conditions were as reported previously (30). To obtain the complete genome of SIVrcmNG411, three additional overlapping fragments spanning PBS/*pol* (4,055 bp), *pol*/*env* (1,915 bp), and *tat*/LTR (3,889 bp) were amplified from genomic DNA, using either the Takara ExTaq kit (Takara, Otsu, Shiga, Japan) for three-step PCR or the Gene Amp XL PCR Kit (PE Applied Biosystems, Branchburg, N.J.) for two-step PCR. PCR conditions and primers were as follows: (i) for *PBS*/*pol*, RCMNM/PBS/F (5′-TGGCGCCCGAACAGGGACTTGA-3′) and RCMNM/*pol*/R (5′-TTTCA CTGGCCATCTGGCTGCTAA-3′), 1 cycle at 94°C for 1 min, 35 cycles at 94°C for 45 s and 65°C for 7 min and 1 cycle at 72°C for 15 min; (ii) for *pol*/*env*, RCMNM/*pol*/F (5′-TACAATCCTCAAAGTCAAGGAGT-3′) and RCMNM/*env*/R (5′-TCCATACTGGGACACCATAGAA-3′), 35 cycles at 94°C for 1 min, 55°C for 90 s, and 72°C for 2 min and 1 cycle at 72°C for 10 min; (iii) for *tat*/*LTR:* RCMNM/*tat*/F (5′-TTCACTCGAGAAACATCTTGTAATA-3′) and RCMNM/LTR/R (5′-ATCGTTCGAACTGGTTGGGATTTTTTCTTAG-3′), 1 cycle at 94°C for 1 min, 40 cycles at 94°C for 30 s and 60°C for 6 min, and 1 cycle at 72°C for 15 min. Restriction enzyme sites are underlined. Amplified fragments were cloned into plasmid vectors pCRII-TOPO (Invitrogen, Carlsbad, Calif.) or pGEM-7Zf (Promega, Madison, Wis.) and sequenced by automated fluorescent sequencing (DNA sequencing kit; PE applied Biosystems, Warrington, United Kingdom). The SIVrcmNG411 proviral sequence was assembled using the program Geneworks (IntelliGenetics); 30 bp at the 5′ end and 3′ end of each partial sequence, which includes the primer sequence, and the C termini at the overlaps were excluded from the assembly.

To obtain the SIVrcmNG409 sequence, PCR products were amplified directly from genomic DNA extracted from frozen red-capped mangabey PBMC. To determine whether the sequence is related to SIVrcmNG411, primers highly conserved between HIV-1, HIV-2, and SIV were used in a nested PCR as described previously (7): These were for the outer primer pair DR1, 5′ TRCA YACAGGRGCWGAYGA 3′, and DR2, 5′ AIADRTCATCCATRTAYTG 3′, and for the inner primer pair DR4, 5′ GGIATWCCICAYCCDGCAGG 3′, and DR5, 5′ GGIGAYCCYTTCCAYCCYTGHGG 3′. The conditions were 40 cycles at 94°C for 15 s, 50°C for 30 s, and 72°C for 1 min and 1 cycle at 72°C for 10 min. Four other fragments of the SIVrcmNG409 sequence were amplified by using SIVrcm-specific or SIVsm/SIVrcm consensus primers: fragment 1 was amplified with primers ExF(1), 5′ CAC TGC TGA TWC AAA ATG CTA A 3′, and ExR(1), 5′ CTG ATA TCT AAT ACC AGG TCC 3′, for the outer set and InF(1), 5′ RCT YAA GGG TCT GGG MAT GAA 3′, and InR(1), 5′ TTC TGA AAG GCT CAT AAA GGG 3′, for the inner set. Fragment 2 was amplified with primers ExF(2), 5′ GTC TCT CCC TGG GAG GCT ACC 3′; ExR(2), 5′ TGC ATG GCT TCT GCC AAT ACT 3′; InF(2), 5′ AGC TTG AGC CTG GGT GTT CGC T 3′; and InR(2), 5′ CTT TAT GCA GAG GCC CTC CTA 3′. Fragment 3 was amplified with primers ExF(3), 5′ CTA GAT ATA GGG GAT GCC TAT 3′; ExR(3), 5′ TGT YTC TGC TGG TAT TAC TTC TGC 3′; InF(3), 5′ GCA TTC ACA ATA CCT GCA ATT 3′; and InR(3), 5′ ATG TGT GCA RTC CAT TTG CCA AGT 3′. Fragment 4 was amplified with primers ExF(4), 5′ GTA GCT CAA TGT CCT AAG TGC CAG 3′; ExR(4), 5′ TCA TGC CAG TGT TCC ACA CAA GT 3′; InF(4), 5′ GAC AGG TAG ATG CCA GTC CAG GAA 3′, and InR(4) 5′ GAA AAT GAA CTC TGG ATG AAA GTG 3′. The conditions were 1 cycle at 94°C for 2 min, 40 cycles at 94°C for 1 min, 55°C for 90 s, and 72°C for 2 min, and 1 cycle at 72°C for 10 min. The PCR products were cloned into the pCRII-TOPO vector (TOPO TA Cloning kit; Invitrogen) and sequenced by automated fluorescent sequencing (*Taq* amplification/termination; Perkin Elmer Applied Biosystems). The SIVrcmNG409 proviral sequence was assembled using the program Geneworks (IntelliGenetics).

Cell lines and virus stocks. Human $CD4^+$ cell lines (MT2, MT4, H9, U937, SupT1, CEMss, CEMx174, PM1, Hut78, and Molt4clone8) were maintained in RPMI 1640, supplemented with 10% heat-inactivated FCS, 2 mM glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 10 mM HEPES. PBMC were separated by Ficoll-Hypaque density gradient centrifugation of whole blood and stimulated with 2 μg of PHA (Sigma) per ml for 3 days and then maintained in complete RPMI, supplemented with 5 half-maximal units per ml of human interleukin-2 (Advanced Biotechnologies, Columbia, Md.). An infectious virus stock of SIVmnd121 was generated by transfecting the plasmid pMD121 (41) into CEMss cells. Virus stocks of other SIVs and HIVs were produced by infecting susceptible cells and harvesting the supernatant at the peak of RT activity, filtering it through a 0.45-μm-pore-size filter and cryopreserving the stocks in the vapor phase of liquid nitrogen. SIVlhoest7, SIVlhoest447, SIVlhoest524, SIVsun, SIVmnd, and SIVagmVer90 virus stocks were produced in CEMss cells. CEMx174 cells were used to grow virus stocks of SIVsmE660, HIV-1 clone 89.6 (subtype B), and SIVsyk and PHA-stimulated

human PBMC for virus stocks of SIVrcmNG411 and HIV-1 DH12 (subtype B). For infection studies, 2.5×10^6 PHA-stimulated human PBMC were infected in 12-well plates (Costar, Corning, N.Y.) and 1×10^7 chimpanzee PBMC in T25 flasks. Virus amounts corresponding to 500,000 cpm of RT activity for human PBMC and to 100,000 cpm of RT activity for chimpanzee PBMC were used. Cells were infected with shaking every 30 min during the first 2 hours. The input virus was not removed but could not be detected in the supernatant by RT assay after 1 to 2 days of incubation with the virus.

GHOST cell assay. The coreceptor usage of SIVlhoest7, SIVlhoest447, and SIVlhoest524, SIVsun, SIVmndGB1, SIVrcmNG411, and HIV-1 clone 89.6 was determined using GHOST(3) (human osteosarcoma) cells (31) which were obtained through the U.S. NIH AIDS Reagent Program. GHOST cells used expressed CD4 only and CD4 in combination with the following coreceptors: CCR2B, CCR3, CCR5, CXCR4, BOB/GPR15, and Bonzo/STRL33. These cells were cultured in complete Dulbecco's minimal essential medium containing G418 (5 μg/ml), hygromycin (1 μg/ml), and puromycin (1 μg/ml). GHOST cells expressing only CD4 served as controls; they were cultured in the same medium except that puromycin was omitted.

For infection experiments, 2×10^5 cells were seeded in 24-well plates (Costar) 2 days prior to infection to obtain a subconfluent cell layer by the time of infection. Equal amounts of virus (500,000 cpm of RT activity) were applied in the presence of 20 μg of polybrene (Sigma) per ml to enhance infection efficiency. Cells were infected for 5.5 h and then washed once with 1× Hanks' balanced salt solution. Samples for RT measurement were taken on days 0, 2, 4, 6, and 9. On days 2, 4, 6, and 9 cells were split 1:2 and subjected to fluorescence-activated cell sorter analysis (FACS) analysis for green fluorescence protein (GFP) expression. To prepare the cells for FACS analysis, GHOST cells were trypsinized for 5 min at 37°C, spun in a microcentrifuge for 30 s at 7,000 rpm, washed once with phosphate-buffered saline, spun again, and resuspended in 4% paraformaldehyde overnight at 4°C. The next day the GHOST cells were resuspended in phosphate-buffered saline–2% FCS and subsequently analyzed for GFP expression on a FACScan (Becton Dickinson, San Jose, Calif.). For RT measurements, supernatants were clarified from cells by short-term centrifugation. RT measurements were performed as described previously (37).

Sequencing of the CCR5 gene. To sequence the CCR5 gene, DNA was extracted from PBMC of 13 red-capped mangabeys. A 0.5-μg amount of genomic DNA was subjected to PCR analysis. The following Primers were used as described previously (27): 5′ GGG GAT CCG GTG GAA CAA GAT GGA T 3′ and 5′ CCC TCG AGC CAC TTG AGT CCG TGT CAC A 3′. The PCR products were cloned into the pCRII vector (TOPO TA Cloning kit) and sequenced by automated fluorescent sequencing (*Taq* amplification/termination; PE Applied Biosystems). To determine internal deletions of the CCR5 gene in red-capped mangabeys, the following primer pairs were used as described previously (6): 5′ GCT CTA TTT TAT AGG CTT CTT CTC TG 3′ and 5′ GTG TAA TGA AGA CCT TCT CTC TGA GAT CTG 3′. Using this primer pair, a 189- or 213-bp fragment of the CCR5 gene was amplified, depending on whether the red-capped mangabey had a deletion in the CCR5 gene. PCR products were separated on a 0.9% agarose gel and visualized by ethidium bromide staining.

Human PBMC were screened for the 32-bp deletion in CCR5 using primers as previously described (26). Amplification products of either 182 or 150 bp were visualized on a 0.9% ethidium bromide-stained agarose gel.

Sequence analysis. The sequences of SIVrcmNG411 and NG409 were aligned and compared to representatives of the major lentivirus lineages including some of those in the HIV sequence database http://hiv-web.lanl.gov/ALIGN_CURRENT/ALIGN-INDEX.html "other SIV complete genome DNA" alignment. Columns in the alignment in which gaps had been inserted to maintain the alignment through regions with insertions and deletions were stripped prior to the analyses. The gap-stripped alignment was first analyzed with the SIMPLOT program (http://www.med.jhu.edu/deptmed/sray/download), both as nucleotide and as translated amino acid sequences, in order to determine the regions of the alignment to be analyzed separately by phylogenetic methods. A neighbor-joining phylogenetic tree was built for each genomic region, using the PHYLIP dnadist and neighbor programs (http://evolution.genetics.washington.edu/phylip.html) with the F84 model of evolution. The resulting tree was used as input, along with the alignment, to Gary Olsen's DNArates program (http://w.w.w.geta.life.uiuc.edu/~gary/programs/DNArates.html) to compute site-specific rates of evolution. These rates were then included in the input to a modified version of fastDNAml, written by Tanmoy Bhattacharya (http://www.santafe.edu/~btk/science-paper/bette.html), which computes maximum-likelihood trees incorporating site-specific rates of evolution. For the first three of the seven trees, the maximum-likelihood tree was used as input to DNArates iteratively, to recalculate the site-specific rates and then rebuild the phylogenetic tree. The resulting trees were nearly identical, both in topology and maximum likelihood scores. Thus, the iteration process was not used on the remaining four trees. Bootstrap support for each of the trees was calculated with the PHYLIP seqboot, dnadist, neighbor, and consense programs. Although the bootstrap trees were computed via the neighbor-joining method, rather than by maximum likelihood with site-specific rates, the topologies were often identical, although branch lengths differed significantly. Trees were edited in TreeTool (http://ftp.cme.msu.edu/pub/RDP/programs/TreeTool/) to produce radial trees and adjust all trees to the same distance scale. RNA secondary structures of TAR were predicted with the RNA MFOLD 3.0 program by M. Zuker and D. Turner at a 37°C folding temperature (http://w.w.w.mfold2.wustl.edu/~mfold/rna/form1.cgi [29, 47]).

Nucleotide sequence accession numbers. The sequences of SIVrcmNG411 and NG409 have been submitted to GenBank under accession numbers AF349680 and AF349681. The red-capped mangabey CCR5 gene sequences have been submitted to GenBank under accession numbers AF349682 and AF349683.

RESULTS

Low seroprevalence of SIVrcm among red-capped mangabeys from Nigeria. Sera from 13 red-capped mangabeys in a monkey sanctuary in Nigeria were investigated for the presence of SIVsm cross-reactive antibodies by radioimmunoprecipitation. Twelve of the mangabeys were individually captured in the wild and came as orphans to the sanctuary, whereas one animal was born in captivity as the offspring of one of the wild-caught mangabeys. Serologic cross-reactivity was observed in sera from two (17%) of the wild-caught red-capped mangabeys, RCM409 and RCM411. As shown in Fig. 1, both sera immunoprecipitated the SIVsm gp160, gp120, and gp41. Serum from RCM411 also immunoprecipitated the SIVsm Gag antigens. RCM409 was also seropositive for simian T-lymphotropic virus (data not shown).

The previously reported SIVrcm isolate from Gabon had replicated in human PBMC (14). Therefore, virus isolation was attempted by cocultivation of PBMC from both seropositive animals with human PBMC. Virus was isolated from PBMC of one mangabey (RCM411), and this isolate was designated SIVrcmNG411. The SIVrcmNG411 isolate was evaluated for coreceptor utilization and replication in various cell lines and primary cells. Total cellular DNA was extracted from virus-producing PHA-stimulated red-capped mangabey PBMC (RCM411) or directly from frozen uncultured red-capped mangabeys PBMC (RCM409) for molecular characterization. Total cellular DNA was also extracted from the PBMC of the other 11 mangabeys for analysis of the CCR5 alleles. As described in Materials and Methods, the complete genome of SIVrcmNG411 and the *gag* and *pol* gene of SIVrcmNG409 were amplified by PCR and sequenced.

Similarity in genomic organization of SIVrcm and SIVsm/HIV-2. The entire genome of SIVrcmNG411 and the *gag* and *pol* gene of SIVrcmNG409 were compared to those of other representative primate lentiviruses. The proviral genome of SIVrcmNG411 was 10,412 nucleotides (nt) in length with a genomic organization similar to those of SIVsm and HIV-2 (long terminal repeat [LTR]-*gag-pol-vif-vpx-vpr-tat-rev-env-nef*-LTR). Specifically, SIVrcmNG411 possessed a *vpx* gene (327 nt) in addition to *vpr* and lacked the *vpu* gene, a pattern found previously only among the members of the HIV-2/SIVsm lineage. The three major forms of genomic organization among SIV strains are shown diagrammatically in Fig. 2. The LTR of SIVrcm (855 nt) was the longest of all primate lentivirus LTRs characterized so far and contained all characteristic features, including two NF-κB sites and two potential Sp-1 binding sites.

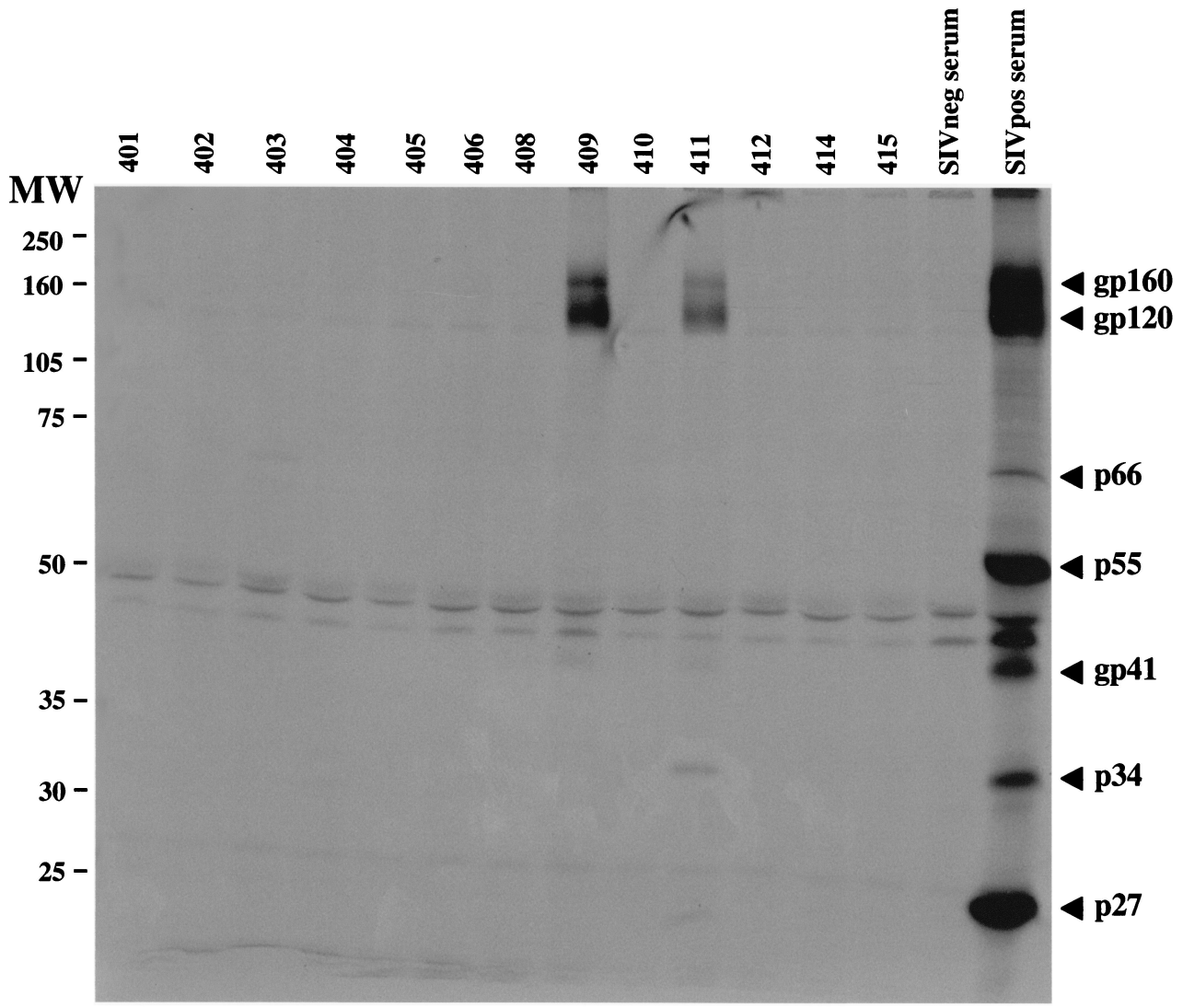


FIG. 1. Serologic identification of SIV infection in wild-caught red-capped mangabeys by radioimmunoprecipitation of SIVsm proteins. Lanes contain SIV antigens immunoprecipitated by plasma samples from 13 red-capped mangabeys and are identified individually by animal numbers; RCM409 and RCM411 plasma samples show a positive reaction with SIVsm envelope proteins. The last two lanes on the right show plasma from an uninfected monkey and plasma from an SIVsm-infected pigtailed macaque. The positions of molecular weight markers (in thousands) are shown to the left, and SIVsm proteins are identified to the right. CEMx174 cells infected with SIVsmE660 were labeled overnight with L-[^{35}S]methionine and L-[^{35}S]cysteine (Amersham), lysed, and precipitated with plasma from wild-caught red-capped mangabeys from Nigeria.

The LTR of SIVrcm was most closely related to the LTR from SIVagmSab, with a 69% nucleotide identity. SIVrcm LTR had also the longest TAR structure of the primate lentiviruses currently characterized (173 bp), followed by SIVagmSab (161 bp). As was observed for SIVagmSab and SIVsm/HIV-2, the stem-loop consensus structure of the TAR region [CU(C)GG (A)GU(A)] and its surrounding nucleotides were duplicated in the SIVrcmNG411 LTR. However, unlike SIVsm/HIV-2, SIVlhoest, and SIVsyk, the energetically most stable structure predicted by RNA secondary structure programs contained only one stem-loop ($\Delta G = -91.9$ kcal/mol; Fig. 3).

Evidence for mosaic genome structure of SIV from red-capped mangabeys. The predicted proteins of SIVrcmNG411 and -NG409 were compared with representatives of the other lentivirus lineages as detailed in Table 1. First, we were interested in the relationship of these new SIVrcm strains to the previously characterized SIVrcmGB1 originating in Gabon. The translated-954 bp *gag* fragment and 475-bp *pol* fragment of SIVrcmGB1 were compared to the analogous regions of SIVrcmNG411 and -NG409. As shown in Table 1, SIVrcmNG411 and -NG409 were closely related to SIVrcmGB1 (87 and 89%, respectively, in Gag and Pol), suggesting that red-capped mangabeys were the natural host of this type of virus. However, consistently with their geographic origins, SIVrcmNG411 and -NG409 were more closely related to each other (96 and 94%, respectively, in Gag and Pol) than to SIVrcmGB1. Unexpectedly, the second most closely related primate lentivirus to SIVrcm was SIV from a drill (SIVdrl; 65% for Gag and 76% for Pol), for which a 3,611-bp region of *gag* and *pol* is now available for comparison (GenBank acces-

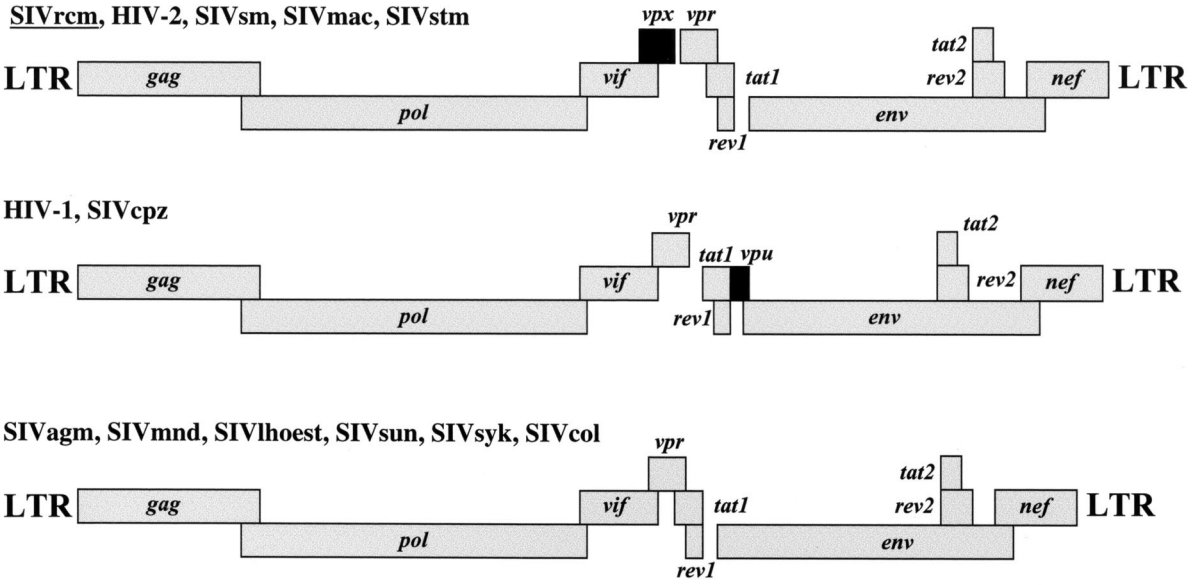


FIG. 2. Genomic organization of SIVrcm compared to other representative primate lentiviruses. The genome organizations are shown schematically for SIVrcm, SIVsm, HIV-2, SIVmac, and SIVstm (top panel), SIVcpz and HIV-1 (middle panel), and SIVagm, SIVsyk, SIVlhoest, SIVsun, SIVmnd, and SIVcol (bottom panel).

sion no. AJ310481 [7]). This close relationship was unexpected since drills and red-capped mangabeys are only distantly related species of monkeys. However, the two species share a common habitat in western central Africa (Nigeria and Cameroon) and also some ecological characteristics, including a diurnal activity cycle and a way of life which is both arboreal and terrestrial. Therefore, a cross-species transmission of viruses at some point in the past could explain this unexpectedly close genetic relationship.

With the exception of partial sequences of SIVagmSab and SIVcpz, all other primate lentiviruses were only distantly related to SIVrcm in Gag and Pol, ranging from 46 to 65% identity. The Gag protein of SIVrcm was most closely related to SIVagmSab (72%). Comparison of the Pol protein revealed that it was also most closely related to SIVcpz and SIVagmSab. SIVrcm showed the highest sequence similarity to the SIVsm lineage in the Vpx/r, Tat, Rev, and Nef proteins, whereas the Env protein was approximately equidistant from SIVsm and SIVagm (both SIVagmSab and SIVagmVer). Despite the shared feature of a *vpx* gene with viruses of the SIVsm/HIV-2 lineage, the predicted Vpx protein of SIVrcm was only distantly related to the Vpx protein of SIVsm (44% identity). These data confirm the earlier impression, based upon partial Gag and Pol sequences of SIVrcmGB1 (14), that SIVrcm cannot be readily classified into a particular primate lentivirus lineage.

To investigate the extent of sequence difference across the genome in more detail, similarity plots of nucleotide sequences of SIVrcm and representatives of each of the primate lentivirus lineages were constructed as shown in Fig. 4 and 5. Since SIVagmSab was known to be a recombinant, we included this virus for comparison as well as SIVagmVer. As expected from the protein comparisons, a close genetic relationship was observed across the *gag* and *pol* gene of SIVrcmNG411 and 409 (Fig. 4). The similarity plot also revealed that the genetic relationship between SIVrcm and SIVdrl was limited to parts of the *pol* gene, mostly the amino-terminal part. The similarity plot across the entire genome between SIVrcmNG411 and representatives of the six primate lentivirus lineages is shown in Fig. 5B. Consistent with the genetic relationship of their natural monkey hosts, SIVrcmNG411 was highly divergent from SIVlhoest, SIVsyk, and SIVcol in all genes. The relative extent of sequence similarity of SIVrcmNG411 to SIVagmSab and SIVcpz varied along the *gag* and *pol* genes. While SIVrcmNG411 was most closely related to SIVagmSab and SIVsmm in *gag* and to SIVagmSab in the central portion of *pol*, it was most closely related to SIVcpzGab in the amino- and carboxy-terminal portions of *pol*. The *env* gene of SIVrcmNG411 was highly divergent from the *env* gene of SIVcpz, showing considerably more similarity to *env* of SIVsmm and the two representative SIVagm clones. Such crossing of similarity plots

TABLE 1. Protein sequence identities among primate lentiviruses

Lentivirus	% Protein sequence identity with SIVrcmNG411 for:								
	Gag	Pol	Vif	Vpx	Vpr	Tat	Rev	Env	Nef
SIVrcmNG409	95 (96[a])	94 (94[a])	–	–	–	–	–	–	–
SIVrcmGB1	87[a]	89[a]	–	–	–	–	–	–	–
SIVdrl	65[b]	76[b]	–	–	–	–	–	–	–
SIVcpz	61	70	45	–	48	33	29	37	53
SIVsmm	63	61	48	44	59	59	57	51	64
SIVagmSab	72	71	51	–	40	40	37	51	60
SIVagm Ver	65	63	43	–	45	43	40	51	54
SIV1hoest	50	58	29	–	40	36	31	35	45
SIVsyk	55	54	36	–	33	52	38	40	47
SIVcol	46	50	21	–	30	36	31	28	31
SIVtal	–[c]	51[d]							

[a] alignment with 954 bp in *gag* and 475 bp in *pol*.
[b] alignment with 948 bp in *gag* and 2,907 bp in *pol*.
[c] –, no comparison possible.
[d] alignment with 550 bp in *pol*.

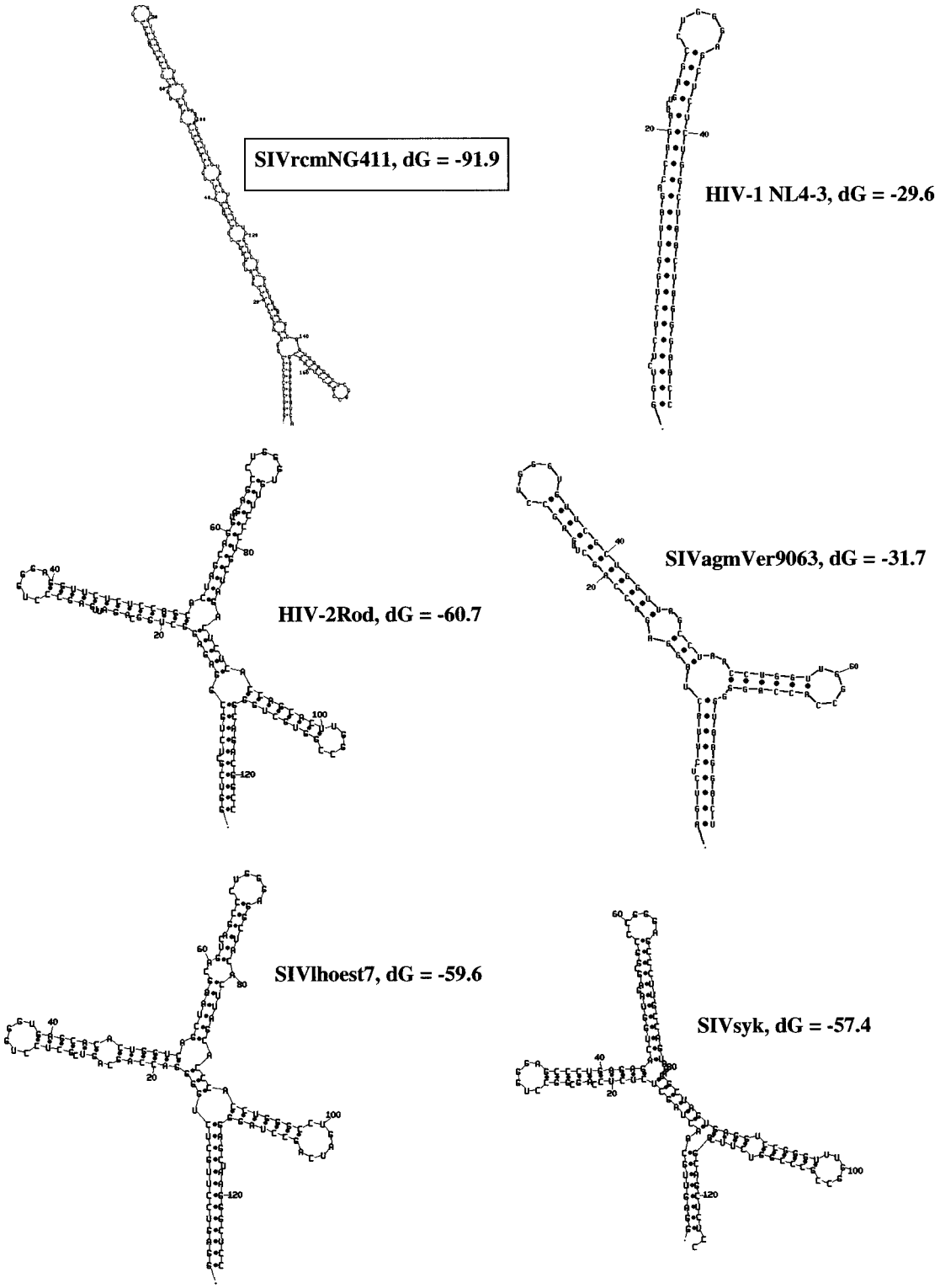


FIG. 3. RNA secondary structures predictions of TAR. Secondary structure predictions of SIVrcm and other primate lentiviruses as generated by the RNA MFOLD 3.0 program by M. Zuker and D. Turner at a 37°C folding temperature (http://w.w.w.mfold2.wustl.edu/~mfold/rna/form1.cgi [29, 47]). The free energy is expressed in kilocalories per mole. The different viral strains are as indicated (for GenBank accession numbers of virus strains, see Material and Methods).

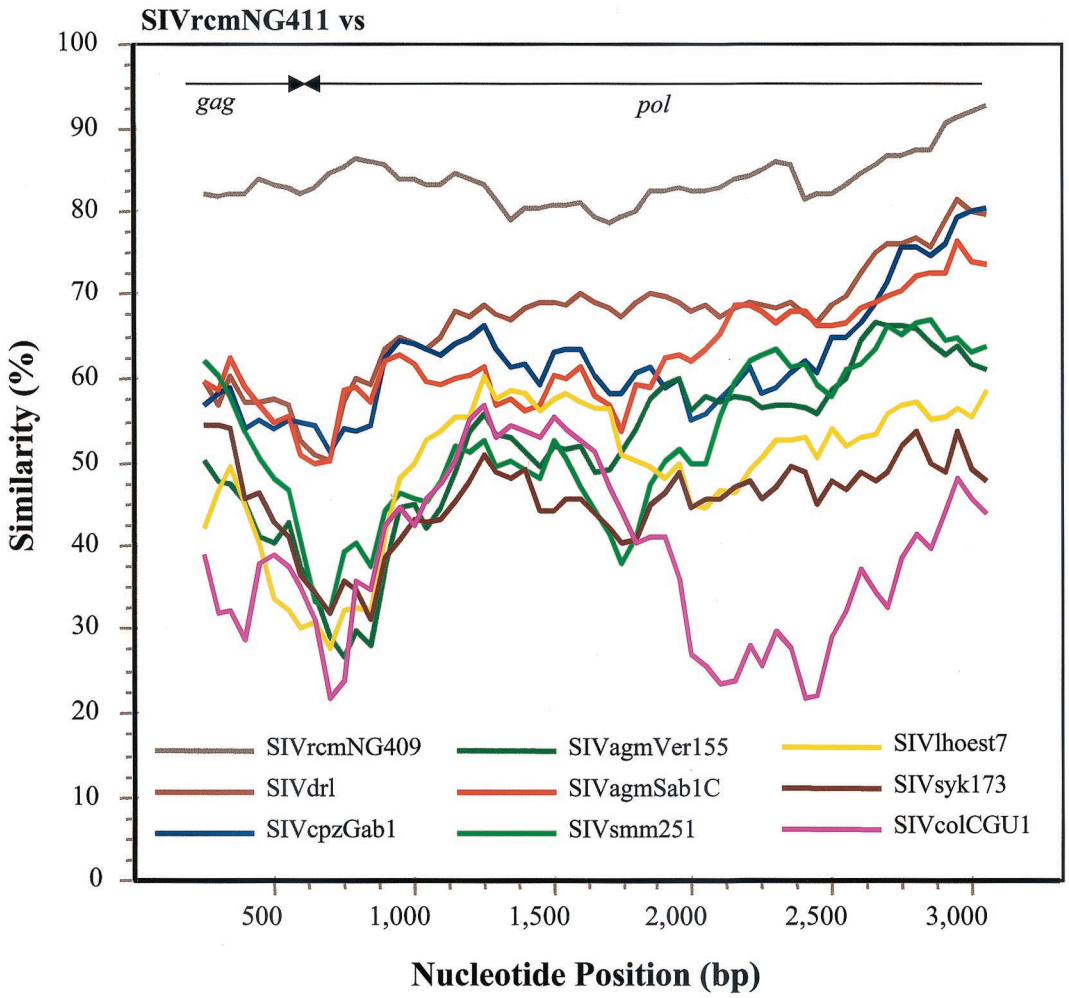
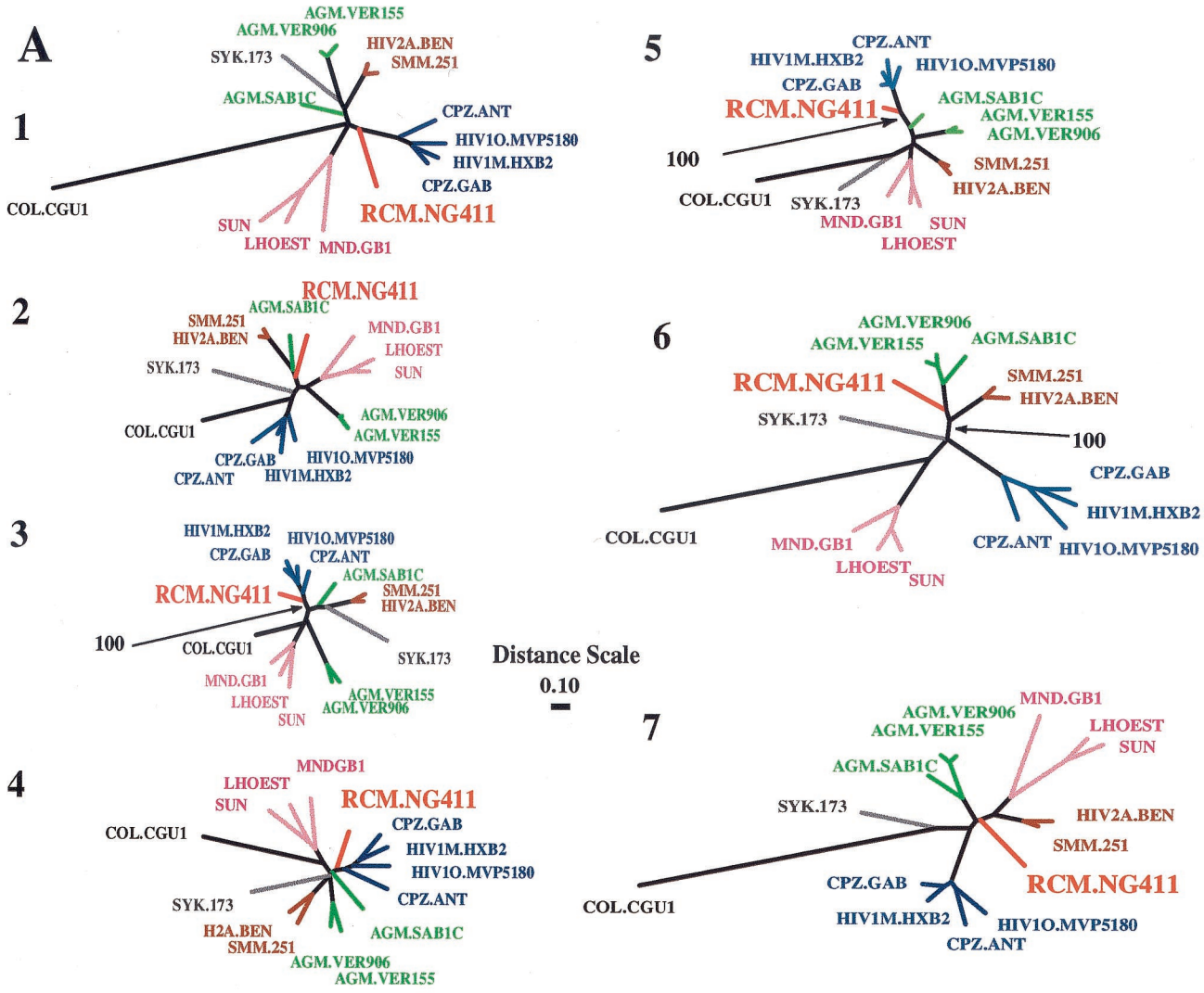


FIG. 4. Similarity plots comparing parts of *gag* and *pol* of SIVrcmNG411 with those of SIVrcmNG409, SIVdrl, and representatives of the six major lineages of primate lentiviruses, SIVcpz, SIVsmm, SIVagmSab, SIVagmVer, SIVlhoest, SIVsyk, and SIVcol.

is generally diagnostic of mosaic genomes generated by recombination, although these patterns can also arise due to relatively short branch lengths between sequences which do not fall on the same lineage. Although the Vpx proteins of SIVrcm and SIVsmm display only 44% identity, SIVrcm was most closely related to SIVsm/HIV-2 and SIVagm in the 3′ region including the *vpx*/*vpr*, *env*, and *nef* genes, consistent with the origin of *vpx* from either SIVsm or SIVagm (43, 44).

Finally, the phylogenetic relationships of the two SIVrcm sequences were estimated by both neighbor-joining and maximum-likelihood analysis. The 6,662-bp gap-stripped alignment of nucleotide sequences was divided into seven regions, according to the crossover points at which the relative relationship of the analyzed viruses to SIVrcmNG411 changed, as indicated in Fig. 5B. For each region, phylogenetic trees were created as described in Materials and Methods. For practical

FIG. 5. Phylogenetic trees and similarity plot analysis of primate lentiviral full-length genomes. (A) The primate lentiviral genomes were aligned as described in Materials and Methods, columns containing gaps were removed from the alignment, and the resulting gap-stripped alignment was subdivided into seven regions based on the similarity relationship of the SIVrcm sequence to other sequences as detected by SimPlot (Fig. 6B). Each region was then used to build a phylogenetic tree as described in Materials and Methods. The sequences used are all available from http://w.w.w.hiv-web.lanl.gov using either the common names or accession numbers (see Materials and Methods). Leaves on each tree are colored similarly to the shades of colors used in the SimPlot. Although many nodes in the trees have 100% bootstrap support, only three nodes (in trees 3, 5, and 6) supported the hypothesis of a recombination event or events involving the lineage leading to the RCM.NG411 sequence, and they are indicated by arrows. In the other four trees, the bootstrap support for RCM.NG411 belonging to a clade including other lineages was less than 50%. (B) The gap-stripped sequence alignment described for panel A was analyzed with the SimPlot program written by Stuart Ray using a window size of 500 bases and a step increment of 20 bases. The nucleotide similarity score computed by SimPlot is a corrected phylogenetic distance/similarity score within the window and corrects for multiple mutations per site via the Kimura two-parameter model, which counts transitions differently from transversions (Ts/Tv ratio set to 1.7 in this figure). Thus, the similarity scores produced by SimPlot can be compared to the phylogenetic distances in panel A, although different models of evolution were used (Kimura 2-parameter in B: F84 maximum likelihood with site-specific rates in panel A).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AGM.VER155
AGM.VER906
HIV2A.BEN
SMM.251
SYK.173
AGM.SAB1C
CPZ.ANT
HIV1O.MVP5180
HIV1M.HXB2
CPZ.GAB
COL.CGU1
SUN
LHOEST
RCM.NG411
MND.GB1
2
RCM.NG411
AGM.SAB1C
SMM.251
HIV2A.BEN
MND.GB1
LHOEST
SYK.173
SUN
COL.CGU1
AGM.VER906
AGM.VER155
CPZ.GAB
HIV1O.MVP5180
CPZ.ANT
HIV1M.HXB2
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HIV1M.HXB2
HIV1O.MVP5180
CPZ.GAB
CPZ.ANT
AGM.SAB1C
RCM.NG411
SMM.251
HIV2A.BEN
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SYK.173
MND.GB1
LHOEST
SUN
AGM.VER155
AGM.VER906
Distance Scale
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MNDGB1
LHOEST
SUN
RCM.NG411
CPZ.GAB
COL.CGU1
HIV1M.HXB2
HIV1O.MVP5180
SYK.173
CPZ.ANT
H2A.BEN
SMM.251
AGM.SAB1C
AGM.VER906
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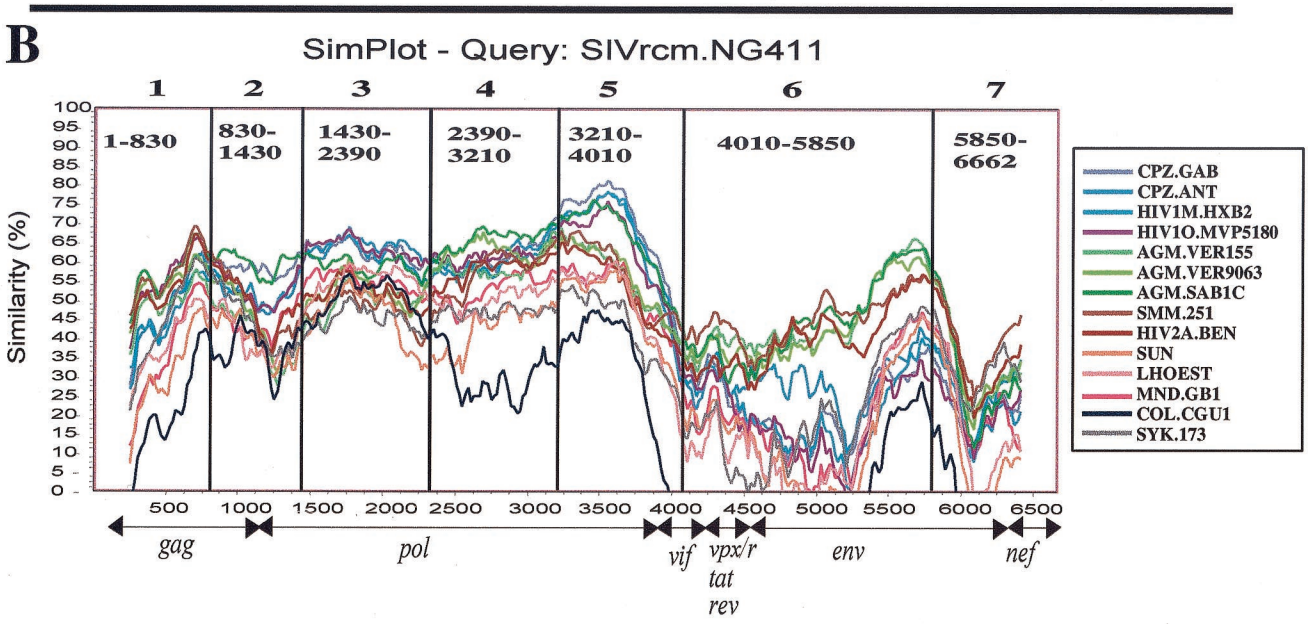
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Similarity (%)
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HIV1O.MVP5180
AGM.VER155
AGM.VER9063
AGM.SAB1C
SMM.251
HIV2A.BEN
SUN
LHOEST
MND.GB1
COL.CGU1
SYK.173

reasons, the 100% bootstrap values for three key nodes are indicated from the neighbor-joining analysis. As expected from the protein identities and similarity plots, this analysis revealed significantly discordant phylogenetic positions of SIVrcmNG411 in relation to other primate lentiviruses in several of the seven different regions (Fig. 5A). In regions 1 (*gag*), 3 (*pol*), and 5 (*pol*/*vif*), SIVrcm could be detected on the HIV-1/SIVcpz branch, although there was less than 50% bootstrap support for that relationship in region 1. In region 2 (*gag*/*pol*), SIVrcm clustered with SIVagmSAB1C and the SIVsmm lineage but with less than 50% bootstrap support. It is noteworthy that SIVcpzGAB-1 was more similar to SIVrcm in this region (Fig. 5B) than other SIVcpz/HIV-1 isolates were, but this seems due to a shorter branch of the GAB-1 isolate rather than to a more recent shared ancestry with SIVrcm (Fig. 5A2). In region 6 (*vpr*, *tat*, *rev*, *env*), SIVrcm grouped with the SIVagm and SIVsmm lineages with 100% bootstrap support, but there was less than 50% bootstrap support for SIVrcm clustering with either the SIVagm or SIVsm lineage. In regions 4 (*pol*) and 7 (*env*/*nef*), SIVrcm formed its own lineage. Only in regions 3, 5, and 6 was the association of SIVrcm with another lentivirus lineage supported by 100% of the bootstrap replicates, as indicated by arrows. Bootscanning, using 500-bp windows, produced comparable results (data not shown). As already suggested by the protein identities and similarity plots, maximum-likelihood phylogenetic analysis with site-specific DNA rates confirmed that the SIVrcm genome is mosaic and potentially the result of several recombination events involving ancestors of viruses circulating in the red-capped mangabey habitat. The genetic relationships between SIVrcm, SIVagmSab, and SIVcpz are quite distant, and the exact history of recombination is thus impossible to decipher. The analysis of the history of recombination responsible for the formation of SIVrcm is complicated by the fact that at least one of its closest relatives, SIVagmSab, is itself a recombinant (23). Hopefully, analysis of more SIV strains, in particular SIVs from drill and mandrill, will elucidate the complex history of evolution of SIVrcm.

Replication of SIVs in human and chimpanzee PBMC: extent of replication is not predictable by viral genome identities. The host range of SIVrcmNG411 was tested in human $CD4^+$ T-cell lines, human PBMC, and chimpanzee PBMC. In terms of human $CD4^+$ T-cell lines, SIVrcmNG411 replicated in PM1, and to a lesser extent in H9 cells. No replication was observed in SupT1, MT4, MT2, U937, Molt4clone8, CEMss, and CEMx174 cell lines (data not shown).

The replication of SIVrcm in human and chimpanzee PBMC was also evaluated in parallel with other SIV strains as shown in Fig. 6. All human donors utilized for PBMC samples were screened for the Δ32-bp deletion in CCR5 and were homozygous wild type (data not shown). As expected from the successful isolation of SIVrcmNG411 in human PBMC, this virus replicated efficiently in human PBMC (Fig. 6A). HIV-1 clone 89.6, SIVsmE660, SIVmndGB1, and SIVlhoest524 also replicated to high titers in both donors. Overall, donor no. 1 was slightly more susceptible to SIV and/or HIV infection than donor no. 2. Two additional SIVlhoest isolates, 447 and 7, also replicated efficiently in human PBMC (data not shown); however, the closely related SIVsun did not replicate in human PBMC. As previously reported, SIVsyk173 and SIVagm90 also did not replicate appreciably in human PBMC (18).

Because SIVrcm is closely related to SIVcpz in the *pol* gene, the replication capacity of SIVrcmNG411 was evaluated in PHA-stimulated chimpanzee PBMC. We hypothesized that if SIVrcm was a recombinant with SIVcpz, then this virus might be capable of replicating in chimpanzee PBMC. HIV-1 DH12, a virus known to replicate in chimpanzee PBMC, served as a positive control. The replication kinetics for two chimpanzee donors are shown in Fig. 6B. SIVrcmNG411 and SIVsmE660 did not replicate in chimpanzee PBMC, whereas SIVmndGB1 and SIVlhoest unexpectedly replicated well in the chimpanzee PBMC cultures. SIVmndGB1 replicated to higher titers than HIV-1 DH12, and SIVlhoest replicated to peak titers similar to those of HIV-1 DH12 and SIVmndGB1, following an initial delay. In summary, we identified SIVs of three lineages capable of replicating in human PBMC: SIVlhoest and SIVmnd (both members of the SIVlhoest lineage), SIVrcm, and SIVsm. Two of these viruses, SIVlhoest and SIVmnd, also replicated in chimpanzee PBMC.

Use of CCR2B and STRL33 is a common feature of SIVrcm. The previously characterized SIVrcm had been demonstrated to utilize CCR2B rather than CCR5 as its major coreceptor. Therefore, the coreceptor usage of SIVrcmNG411 was determined using the GHOST cell assay with a focus on the major coreceptors CCR2B, CCR3, CCR5, CXCR4, STRL33 (Bonzo), and GPR15 (Bob) (Fig. 7A). SIVmndGB1 and HIV-1 clone 89.6 were included as controls since both of these viruses have been reported to utilize CXCR4 and HIV-1 clone 89.6 has also been reported to utilize CCR3 (40, 42). To confirm virus replication in individual target cells, supernatants were assayed for RT activity each time the cells were investigated for GFP expression. Replication capacity in GHOST cell lines, as assessed by supernatant RT activity, correlated with GFP expression (Fig. 7B). Figure 7A shows the percentage of each GHOST cell line expressing GFP at 2, 4, 6, and 9 days following infection with SIVrcmNG411, SIVlhoest, SIVsun, SIVmndGB1, or HIV-1 clone 89.6. As noted in previous reports on SIVrcmGB1, SIVrcmNG411 used CCR2B and STRL33 but did not utilize CCR3, CCR5, CXCR4, or GPR15. In contrast, SIVlhoest (one isolate shown) and SIVsun used CCR5, GPR15, and STRL33. As previously reported, SIVmndGB1 used CXCR4 but not CCR5, GPR15, or STRL33 and HIV-1 clone 89.6 used CCR5, CXCR4, and CCR3.

High allelic frequency of CCR5 Δ24-bp deletion in Nigerian red-capped mangabeys. To determine the frequency of the Δ24-bp deletion in Nigerian red-capped mangabeys, we used diagnostic primers to amplify a CCR5 fragment that spans either a 189- or a 213-bp fragment as previously described (6). As shown in Fig. 8, 8 of the 13 red-capped mangabeys investigated were homozygous and 4 were heterozygous for the CCR5 deletion. One mangabey was homozygous for wild-type CCR5. This corresponds to an allelic frequency of 76.9% for the deleted allele of CCR5. The animal (RCM411) infected with SIVrcmNG411 was heterozygous, and the animal (RCM409) infected with SIVrcmNG409 was homozygous for the Δ24-bp deletion in CCR5. To investigate the sequence of CCR5 and the exact location of the deletion, the entire sequence of red-capped mangabey CCR5 from one homozygous (RCM402) animal and one heterozygous (RCM411) CCR5

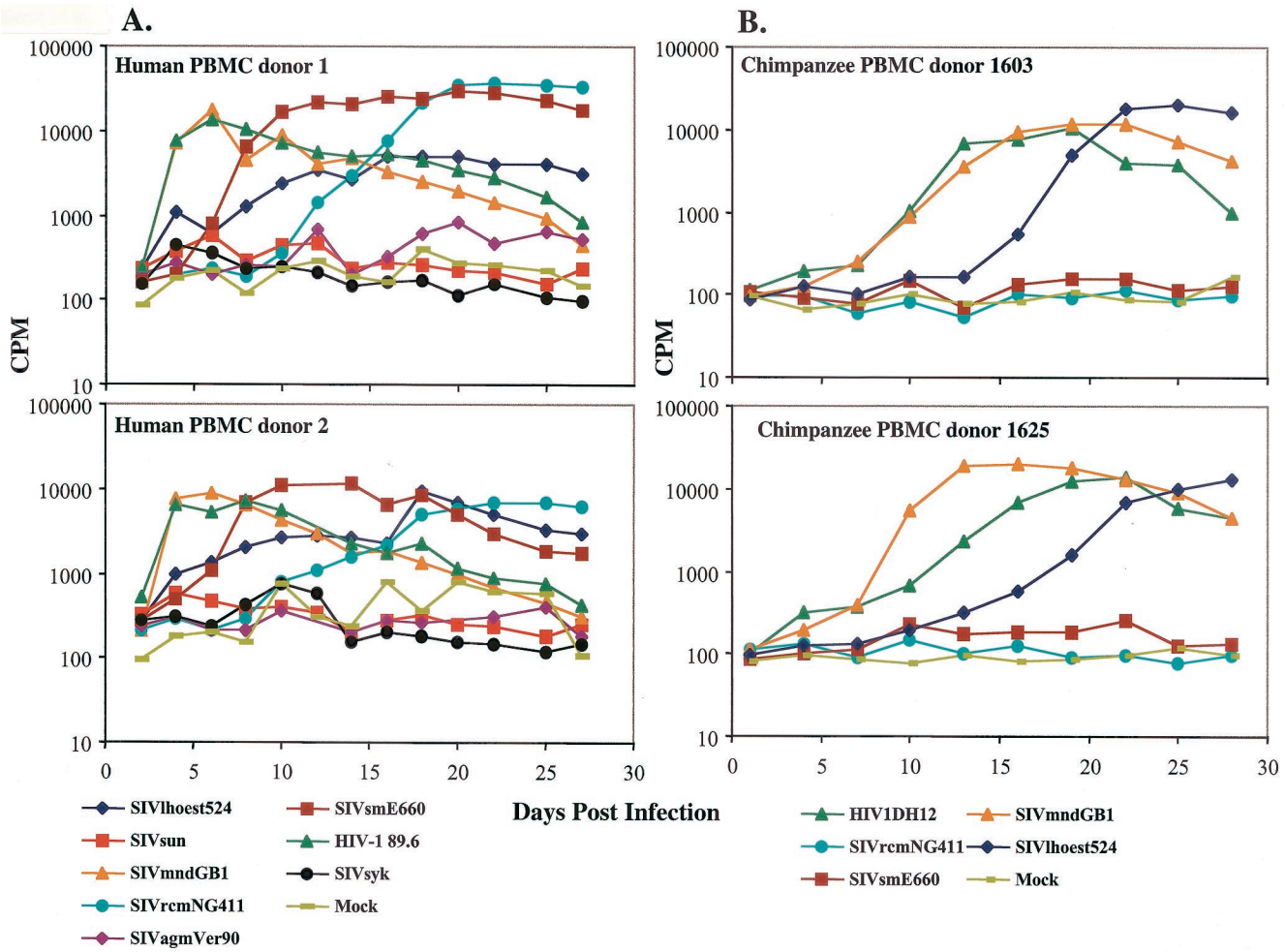

FIG. 6. Replication of SIVrcm in human and chimpanzee PBMC. (A) Infection of PHA-stimulated human PBMC from two different donors with a lack of the 32-bp deletion in CCR5. A total of 2.5×10^6 PHA-stimulated human PBMC were infected in 12-well plates with a virus amount corresponding to 500,000 cpm of RT activity. (B) Infection of PHA-stimulated chimpanzee PBMC from two different donors. A total of 1×10^7 cells were infected in T25 flasks with a virus amount corresponding to 100,000 cpm of RT activity. Virus stocks used are as indicated.

wild-type animal were amplified and cloned (27). The predicted wild-type red-capped mangabey CCR5 protein showed 97.4% identity to the human CCR5 protein. The deletion was found to be 24 bp (439 to 462 bp) as previously described for red-capped mangabeys from Gabon and an American zoo (6). In addition, all wild-type red-capped mangabey CCR5 sequences encoded a proline at amino acid position 180, similar to the CCR5 of sooty mangabeys, whereas the deleted CCR5 allele encoded a serine at that position.

DISCUSSION

Previous studies identified a novel SIV isolate from naturally infected red-capped mangabeys in Gabon that was subsequently designated SIVrcmGB1 (14). Based upon partial sequence analysis, the closest relative of SIVrcm was SIVcpz rather than SIVsm from the more closely related primate species, sooty mangabeys (*Cercocebus atys*). SIVrcm also had the unusual property of utilizing CCR2B rather than CCR5 as its primary coreceptor for viral entry. Genetic analysis of the CCR5 alleles of red-capped mangabeys revealed a high prevalence of an allele with a 24-bp deletion in these populations, suggesting that the use of CCR2B by SIVrcm may have evolved due to the absence of a functional CCR5 gene (6).

We tested 12 wild-caught red-capped mangabey orphans from southeast Nigeria and, as expected, the seroprevalence of these animals was low, because most SIV infections are acquired after sexual maturity in the wild (12, 25, 39); only 2 of 12 (17%) wild-caught animals were SIV positive. Both SIV-positive redcapped mangabeys were brought to the monkey sanctuary, after being confiscated by Nigerian wildlife officials, when they were about 8 months to 1 year old (estimate from body weight and tail length). Since they were housed solely in an enclosure with SIV-negative red-capped mangabeys, they must have become infected with SIV in the wild. Prenatal and perinatal infections are not investigated for African primates except for African green monkeys, in which prenatal and perinatal infections have never been detected (36). Although vertical infections cannot be totally excluded for red-capped mangabeys, the most likely route of transmission before sexual maturity is through biting injuries.

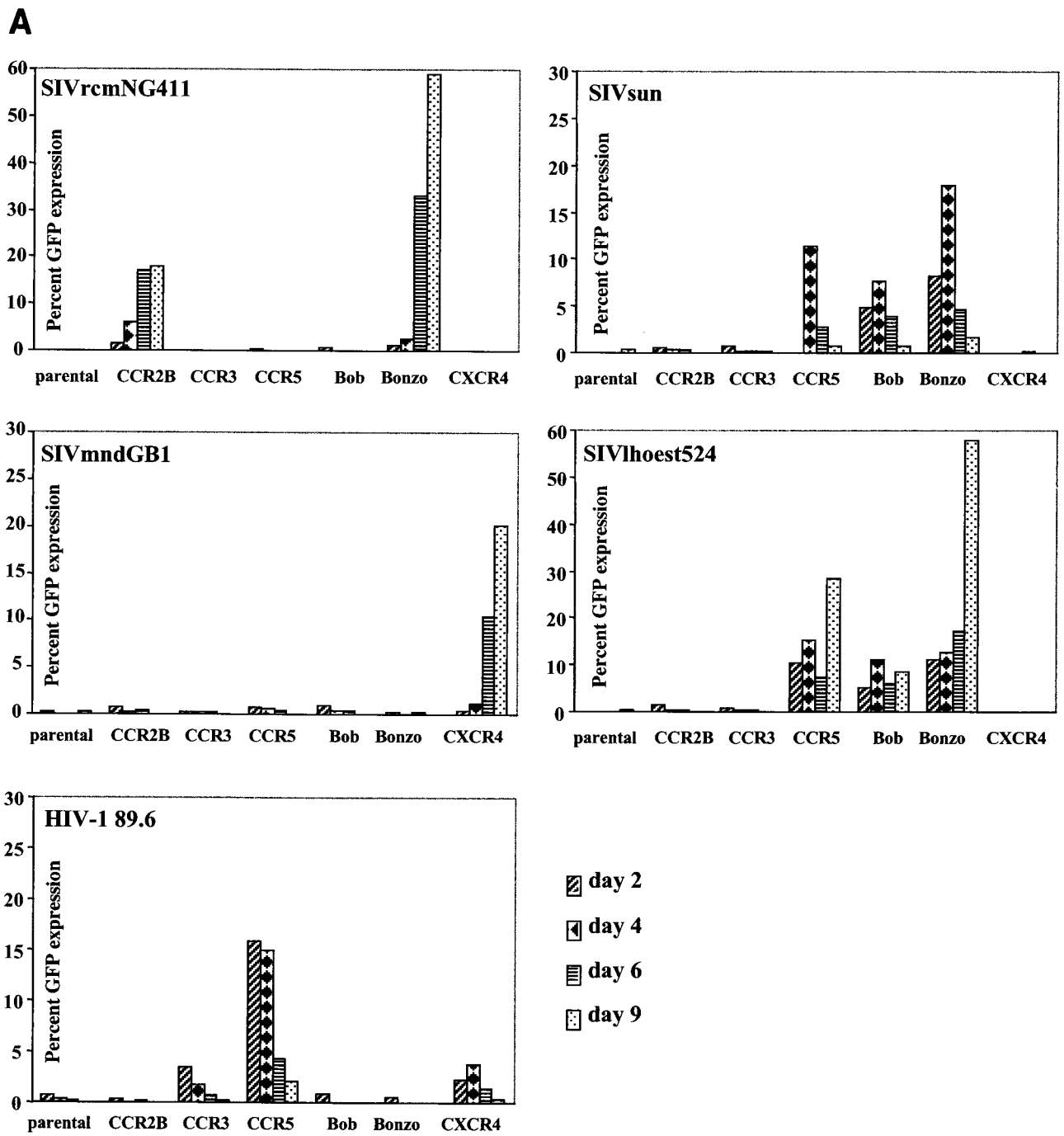

FIG. 7. Evaluation of coreceptor usage of SIVrcm in the GHOST cell assay. Human osteosarcoma cells, transfected with HIV-2 LTR-GFP, CD4, and different HIV coreceptors were infected with SIV or HIV in the presence of polybrene as indicated. (A) GFP expression following transactivation by *tat* was measured on days 2, 4, 6, and 9 after infection. The green fluorescence is quantified as percent GFP-positive cells. The parental GHOST cell line, expressing CD4 only, served as a negative control. (B) The course of infection of GHOST cell lines as monitored by RT activity in culture supernatants.

In the present study, we identified SIVrcm infection in red-capped mangabeys from Nigeria. Sequence analysis of one entire SIVrcm strain (SIVrcmNG411) and one partial SIVrcm strain (SIVrcmNG409) from Nigeria revealed that they were most closely related to the previously characterized SIVrcmGB1 from Gabon (SIVrcmGB1). This suggests that this SIV lineage is naturally circulating in red-capped mangabeys in the wild and that these animals are the natural host for SIVrcm. The two Nigerian sequences were more closely related to one another than to SIVrcmGB1, consistent with the geographic origins of the three strains. SIVrcm shared the genome organization characteristic of viruses of the SIVsm/HIV-2 lineage;

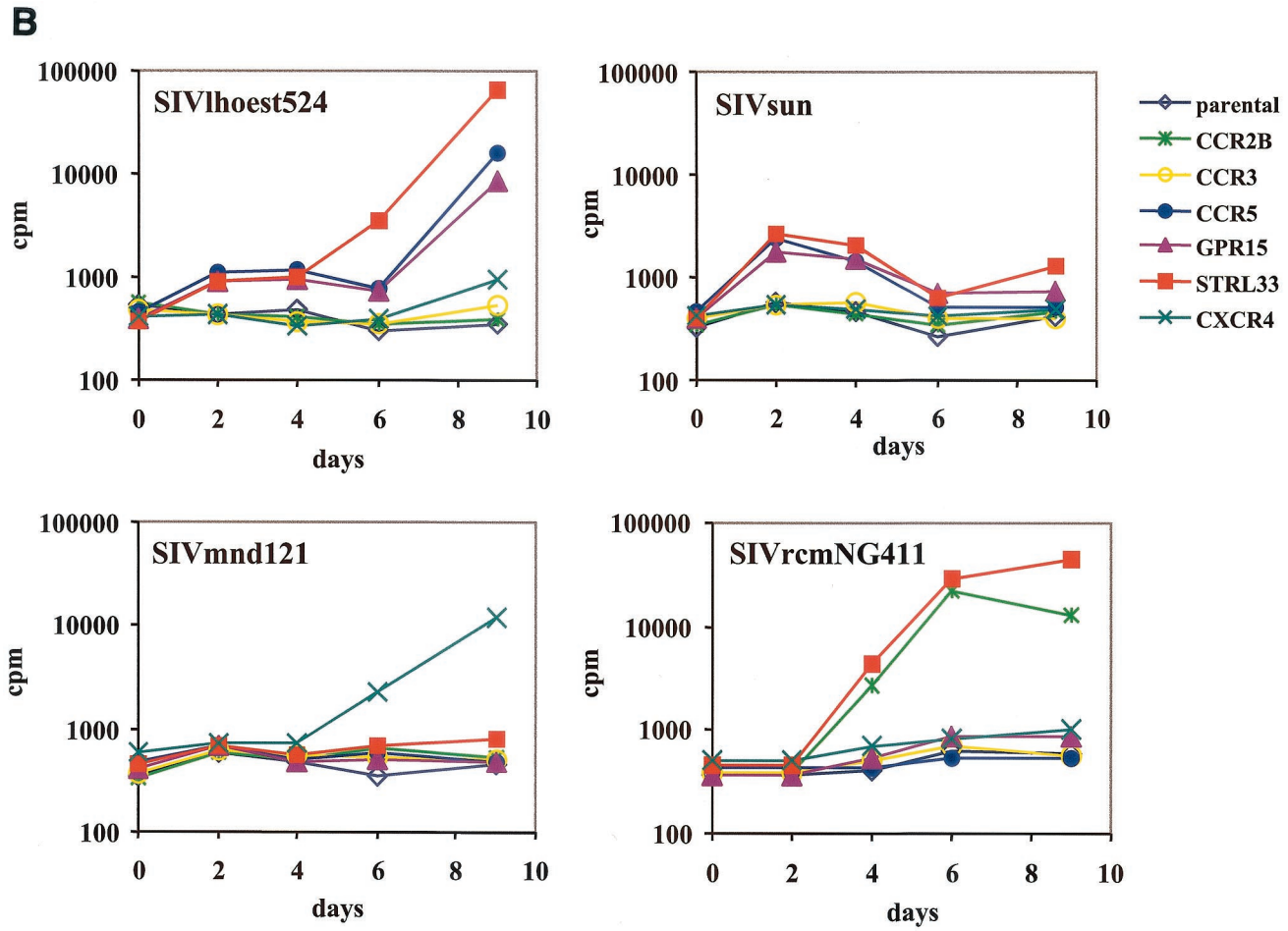

FIG. 7—*Continued.*

each of these viruses possesses a *vpx* gene in addition to a *vpr* gene. The presence of the *vpx* gene in SIVrcm suggests a common evolutionary ancestor with SIVsm. This is not an unexpected finding, since sooty mangabeys and red-capped mangabeys are phylogenetically closely related primate species. However, SIVrcm was quite divergent from SIVsmm across the whole genome and showed only a marginally closer relationship to SIVsmm than that of the other lentiviruses in the *vpx*/*vpr* gene region. Although the identity between the Vpx protein of SIVsmm and SIVrcm was only 44%, they formed a monophyletic cluster in a tree of aligned Vpx and Vpr protein sequences (data not shown). In the *pol* gene, SIVrcm showed significant identity with SIVdrl isolated from a drill, a primate that is phylogenetically distinct from the mangabey monkeys but shares the same habitat with red-capped mangabeys in Nigeria and Cameroon (16). This discordance between sympatric species of origin and the phylogenetic relationship of the SIV strains they harbor is suggestive of cross-species transmission of primate lentiviruses between various primate species in the past. Phylogenetic and similarity plot analyses revealed that SIVrcm clustered with the other primate lentiviruses discordantly in different regions of the genome, suggesting a history of recombination between SIVcpz and SIVagm from the sympatric sabaeus monkey. However, the genetic relationships between SIVrcm, SIVcpz, and SIVagmSab are not very close, indicating that the exact recombination pattern might be masked by evolution of the sequences after the recombination event(s), which might have happened centuries, if not millennia ago. The analysis is also complicated by the fact that SIVagmSab is itself a recombinant.

In addition to sequence similarity, SIVrcmNG411 also shared biological features with SIVrcmGB1 (the isolate from Gabon). Both of these viruses replicated in human PBMC and utilized human CCR2B and STRL33 as their primary coreceptors, rather than CCR5. The red-capped mangabey from which SIVrcmNG411 was isolated still had one intact CCR5 allele. The allelic frequency of the 24-bp deletion in the fourth transmembrane region of CCR5 was 76.9% in wild-caught Nigerian red-capped mangabeys, similar to the allelic frequency seen for red-capped mangabeys from Gabon and an American zoo (6). Since Gabon and Nigeria represent the southern and northern extremes, respectively, of the red-capped mangabey habitat, it can be assumed that the allelic frequency of the deletion is approximately constant throughout the habitat.

The replication of SIV strains in human PBMC could be a predictor of their potential to infect humans. In this study, we confirmed that SIVsm, SIVlhoest, and SIVrcm replicated efficiently in human PBMC. SIVmnd also was observed to effi-

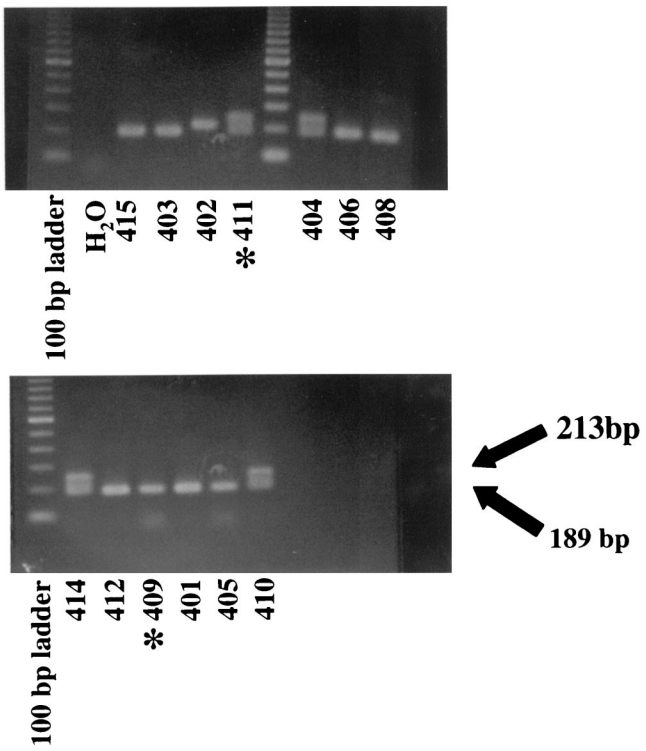

FIG. 8. Analysis of CCR5 alleles of RCM. Separation of PCR products generated with primers spanning a 24-bp deletion in red-capped mangabey CCR5 (deletion from base pairs 439 to 462) in a 0.9% agarose gel. A fragment of 213 bp is indicative of the wild-type CCR5 allele, and a 189-bp fragment is indicative of CCR5 with a 24-bp deletion. Amplification of both fragments indicates heterozygosity for the CCR5 deletion. SIV-positive animals are indicated with stars.

ciently infect and replicate in human PBMC, a finding which has not been previously reported. Surprisingly, SIVsun, which is genetically related to SIVlhoest, did not replicate in human PBMC, suggesting that the ability to replicate in human PBMC can vary within a lineage. Replication in human PBMC was not necessarily predictive of the abilities of these viruses to infect PBMC from the closely related chimpanzee. However, two of the viruses identified as able to replicate in human PBMC (SIVmnd and SIVlhoest) also replicated in chimpanzee PBMC. SIVrcm did not replicate appreciably in chimpanzee PBMC, despite a closer genetic relationship of SIVcpz to SIVrcm than to SIVmnd. SIVcpz and SIVsm have already been introduced in the human population as HIV-1 and HIV-2, respectively. Presumably, SIVmnd, SIVlhoest, and SIVrcm have the potential for similar cross-species transmission scenarios.

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