tical information on the occurrence frequency of REP events is sparse, such events appear to be more prevalent at night. The average occurrence rate probably amounts to at least one event each day. It therefore seems fair to conclude that radiation belt REP may provide a significant natural sink for upper stratospheric O_3.

One must, however, recall that the O_3 destruction associated with REP (and solar proton) events will be primarily confined to altitudes above 30 km. This natural sink for upper stratospheric O_3 can be expected to be most pronounced (implying minimum O_3 content) during solar maximum conditions. Such a behavior makes the observed solar cycle variation of stratospheric O_3 (*16*) even more difficult to understand, since it is anticorrelated with the anticipated response of lower stratospheric O_3 to GCR (*17*). It is therefore clear that detailed models of the vertical transport of NO will be required in order to assess the ultimate response of terrestrial O_3 to variations in particle precipitation over the solar cycle.

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Oncornavirus: Isolation from a Squirrel Monkey (*Saimiri sciureus*) Lung Culture

Abstract. *An oncornavirus isolated from a squirrel monkey (*Saimiri sciureus*) lung culture has a density of 1.16 to 1.17 grams per milliliter, contains 70S RNA, and has an RNA-directed DNA polymerase that prefers Mg^{2+} over Mn^{2+} in an assay in which polyribocytidylate · oligodeoxyguanylate (12–18) is used as a synthetic template. Morphologically, the virus resembles Mason-Pfizer monkey virus but is antigenically distinct from this virus. The virus grows in cells of human, chimpanzee, rhesus monkey, canine, and mink origin, but not cells of squirrel monkey origin. On the basis of its properties, the newly isolated virus can be classified as a retravirus.*

A number of oncornavirus isolates have been recovered from tumors and various "normal" cell cultures of avian and mammalian tissues. Not all members of this group of viruses have been shown to produce cancer in vivo or cell transformation in vitro, but their common properties have led to the proposal that they should be grouped, along with slow and foamy viruses, in the family *Retraviridae* (*1*). Members of this family have enveloped virions (about 100 nm in diameter) that mature by budding from cytoplasmic membranes. They contain single-stranded RNA, an antigenically specific RNA-directed DNA polymerase (RDDP), and several proteins showing different degrees of antigenic specificity. The oncornaviruses, on the basis of morphological criteria, have been placed in four genera, oncornavirus A, B, C, or D. A number of these viruses are endogenous and may (ectropic) or may not (xenotropic) grow in cells of their natural host. An endogenous, xenotropic primate type C oncornavirus has been isolated from fetal and postnatal baboon (*Papio cynocephalus, P. hamadryas*) tissues, including the placenta (*2*).

Although type C viral particles have been observed in other primate placentas, including human, attempts to propagate these viruses in culture have been unsuccessful (*3, 4*). Mason-Pfizer monkey virus (M-PMV), type species, for the candidate oncornavirus D group, has been isolated from the rhesus monkey (*Macaca mulata*) placenta and other fetal and postnatal tissues, including a mammary tumor (*5*). Both the baboon and rhesus are Old World monkey species. Although type C virus particles have been observed under the electron microscope in cebus and marmoset tissues (*4, 6*) and a type C virus, woolly monkey sarcoma virus, was isolated from a woolly monkey tumor (*7*), there have been no previous reports of a type D oncornavirus in tissues of these or any other New World monkey species. We are now reporting the isolation of an oncornavirus with properties similar to M-PMV from a squirrel monkey lung-cell culture.

Lung tissue was obtained from a stillborn, term, male squirrel monkey (*Saimiri sciureus*) shortly after delivery. The tissue was finely minced, trypsinized at room temperature and the cells were suspended in Eagle's minimal essential medium containing fetal bovine serum (10 percent) and antibiotics (100 units of penicillin, 100 μg of streptomycin, 1 unit of bactracin, and 100 μg of neomycin per milliliter). After three passages of the resulting cell outgrowth, attempts were made to isolate an endogenous virus by cocultivating the squirrel monkey lung cells (SqMLu) with a fetal canine thymus culture (FCf2Th) (*8*), a continuous dog kidney cell culture (MDCK), or a diploid chimpanzee fetal lung culture

Table 1. Divalent cation and template preference for viral DNA polymerase (*10*). Results are expressed as 10^3 counts per minute. The figures in parentheses represent the ratio of Mg^{2+} to Mn^{2+} results.

Virus	Poly(rA) · oligo(dT)$_{12-18}$		Poly(dA) · oligo(dT)$_{12-18}$		Poly(rC) · oligo(dG)$_{12-18}$	
	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mn^{2+}
SMRV	636.6 (2.3)	279.2	69.0 (1.8)	39.4	519.7 (39.4)	13.2
M-PMV	75.7 (4.5)	16.8	7.6 (1.9)	3.9	142.0 (25.2)	5.6
M7	5.7 (0.02)	315.9	2.1 (0.6)	3.9	6.0 (0.9)	6.8
RLV	10.9 (0.02)	527.5	5.9 (1.9)	3.2	12.9 (0.3)	44.8

(SFRE:CL-1) (*9*). Concomitantly, SqMLu cells were treated with iododeoxyuridine (IdU) (25 μg/ml) for 4 days prior to cocultivation.

Cultures were split weekly by trypsinization, and culture fluids were tested 7 days after the fourth passage in cocultivation for the presence of RDDP activity (*10*). At this time, polymerase was detected in cocultures with and without IdU treatment, but not in SqMLu, FCf2Th, MDCK, or SFRE:CL-1 cultures alone. The RDDP activity has been detectable through more than 20 passages of SqMLu-FCf2Th coculture, without detectable cytopathology, an indication of the persistent nature of the virus-cell interaction. Electron microscopic examination of RDDP positive cultures revealed intracytoplasmic type A virus particles (60 to 75 nm in diameter) that appeared to migrate to the plasma membrane and bud extracellularly (Fig. 1a). Although crescent-shaped budding structures similar to those found with type C oncornaviruses were occasionally seen (Fig. 1b), there were no mature type C particles. Immature extracellular virus particles (100 to 120 nm) had an electron-lucent core surrounded by an electron-opaque layer and a less opaque layer (Fig. 1c), which was separated from the surrounding envelope by a space. Mature virus (100 to 140 nm) had a centrally located, electron-opaque nucleoid, which was generally spherical but occasionally appeared rod-shaped. This was separated from the envelope by an electron-opaque intermediate layer with an electron-transparent space on either side of it (Fig. 1e). Spikes were not observed on the envelope at any stage of development, nor did the nucleoid appear to be eccentric. When virus purified on a sucrose gradient was negatively stained with phosphotungstic acid (*p*H 7), virus particles displayed typical head-tail configurations without envelope spikes (Fig. 1d). In general, the squirrel monkey virus morphologically appeared to resemble M-PMV (*5*, *11*).

The virus, detected by RDDP activity, banded at a density of 1.16 to 1.17 g/ml in a 12 to 60 percent sucrose gradient. The cation and synthetic template preference of the squirrel monkey virus polymerase was compared to that of M-PMV, baboon endogenous type C virus (M7), and Rauscher leukemia virus (RLV) (Table 1). All of the viruses showed a marked preference for ribopolyadenylate · oligodeoxyribothymidylate (12–18) [poly(rA) · $oligo(dT)_{12-18}$] over polydeoxyriboadenylate · oligodeoxyribothymidylate (12–18) [poly(dA) · $oligo(dT)_{12-18}$] which is indicative of a viral RNA–directed DNA polymerase (*12*). The squirrel monkey virus and M-PMV could only effectively utilize polyribocytidylate · oligodeoxyriboguanylate (12–18) [poly(rC) · $oligo(dG)_{12-18}$] in the presence of Mg^{2+} but not Mn^{2+}. These two viruses also show a preference for Mg^{2+} when poly(rA) · $oligo(dT)_{12-18}$ was used as a template, whereas type C viruses M7 and RLV prefer Mn^{2+}.

Table 2. Host range of the squirrel monkey lung viral isolate. The assay is described in (*10*). Results are given as 10^3 counts per minute of ^{3}H-labeled thymidine monophosphate incorporated. Poly(rA) · $oligo(dT)_{12-18}$ was used as template.

Host cell culture		RDDP assay (10^3 count/min)			
		Control cultures		Inoculated cultures	
Source	Cell line	Mg^{2+}	Mn^{2+}	Mg^{2+}	Mg^{2+}
Canine thymus	FCf2Th	4	5	530	104
Canine kidney	MDCK	2	5	528	209
Human fetal fibroblasts	HSO410	5	4	92	93
Human rhabdomyosarcoma	A204	2	4	35	23
Chimpanzee fetal lung	SFRE:CL-1	3	5	56	41
Rhesus monkey foreskin	rhfs-2	2	3	49	92
Baboon fetal skin	BFS	3	5	2	4
Owl monkey kidney	OMK	2	3	3	5
Howler monkey skin	HMS	4	6	4	4
Squirrel monkey lung	SqMLu	2	3	2	6
Marmoset kidney	MAK	10	5	8	5
Mink lung	MvlLu	5	11	238	285

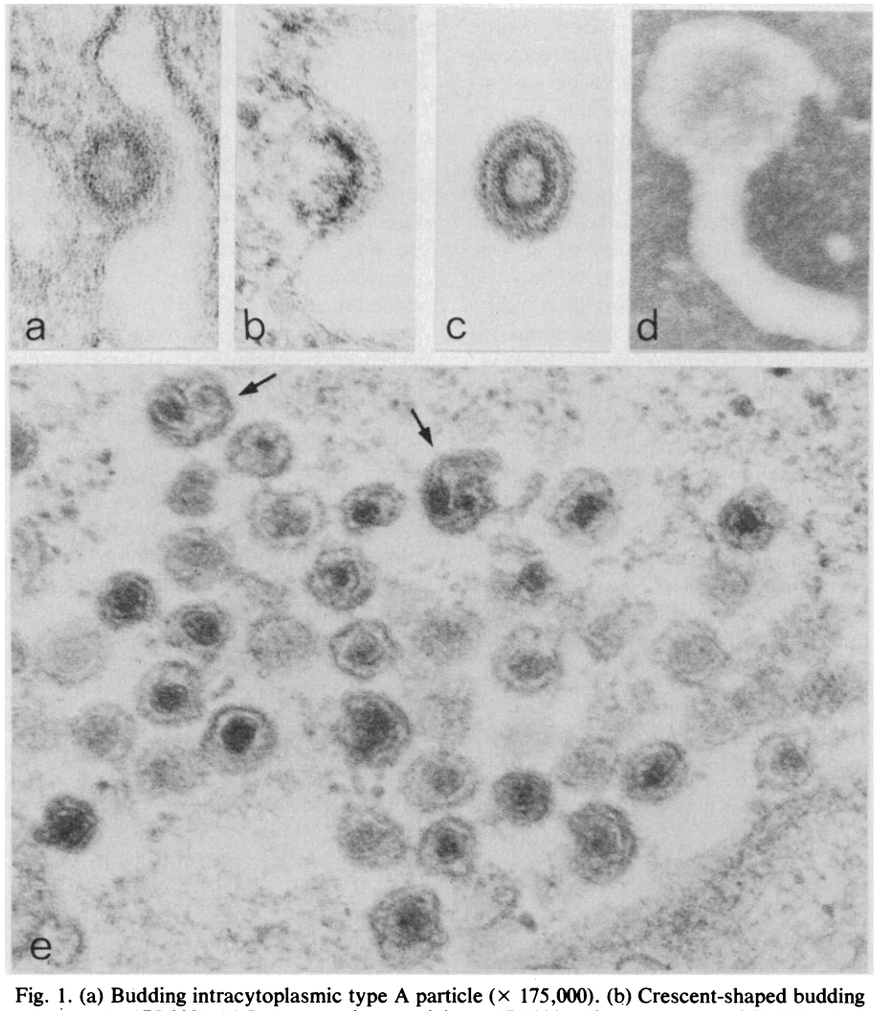

Fig. 1. (a) Budding intracytoplasmic type A particle (× 175,000). (b) Crescent-shaped budding structure (× 175,000). (c) Immature virus particle (× 175,000). (d) Appearance of SMRV when stained with phosphotungstic acid (× 175,000). All preparations were fixed in glutaraldehyde, and then in osium tetroxide; sections were stained with uranyl acetate and lead citrate. (e) Many mature viruses. The arrows indicate double nucleoids in two viruses (× 78,000).

It is probable that the higher polymerase activity noted for M-PMV with poly(rC) · oligo$(dG)_{12-18}$ compared to poly(rA) · oligo$(dT)_{12-18}$ in contrast with the findings of Abrell and Gallo (*13*), is related to differences in KCl concentration in the reaction mixtures employed (*10, 13*).

Further biochemical characterization of squirrel monkey virus, by means of the simultaneous detection technique of Schlom and Spiegelman (*14*), showed the presence of a 70*S* RNA. In addition, tritiated uridine was incorporated into particles banding at a density of 1.16 to 1.17 g/ml.

Although the squirrel monkey lung virus is similar to M-PMV in its morphological and biochemical properties, initial studies indicate that it is antigenically distinguishable from M-PMV. The goat antiserum to M-PMV did not neutralize the infectivity of this virus (*15*). Indirect immune electron microscopy, with horseradish peroxidase labeled rabbit antiserum to goat serum IgG and the same goat antiserum to M-PMV, showed coating of M-PMV particles with antibody, but no reaction was found with the squirrel monkey virus (*16*). Rabbit antiserum prepared against the M-PMV RDDP (*17*) partially inhibited the squirrel monkey polymerase (40 percent inhibition with 10 μg of rabbit IgG compared to 94 percent inhibition of the M-PMV polymerase). Therefore, we feel the squirrel monkey lung virus is distinct from M-PMV.

In a host range study, the squirrel monkey virus was inoculated into a variety of cell cultures (*8*). These included FCf2Th, MDCK, human fetal fibroblasts, human rhabdomyosarcoma, SFRE:CL-1, rhesus monkey foreskin, baboon (*Papio cynocephalus*) fetal skin, owl monkey (*Aotus trivirgatus*) kidney, howler monkey (*Alouatta palliata*) skin, marmoset (*Saguinus fuscicollis*) kidney, mink lung, and SqMLu; the SqMLu culture was the source of the squirrel monkey virus. RDDP activity was detected in culture fluids from inoculated canine, human, chimpanzee, rhesus, and mink cells, but it was not detected in the squirrel monkey lung that was the source of the squirrel monkey virus (Table 2). Cocultivation of infected FCf2Th cells with KC cells resulted in syncytium formation (*18*).

The low ratios of Mg^{2+} to Mn^{2+} obtained from the human, rhesus, and mink cultures inoculated with the squirrel monkey virus may be the reflection of a type C virus, as well as a M-PMV–like virus in the isolate (Table 2). As was noted earlier, however, extracellular type C virus has not been detected in electron microscopic studies. The relatively low activity with Mn^{2+} where poly(rC) · oligo$(dG)_{12-18}$ is used as a template also indicates the absence of type C virus. The occurrence of occasional type C virus buds has also been reported for M-PMV. When these cultures were incubated at 40°C, a transitory increase in these budding type C virus particles was observed (*19*). However, this was not associated with an increase in extracellular type C virus. Whether a similar phenomenon is associated with the squirrel monkey virus remains to be determined.

The properties of the squirrel monkey virus resemble those of the genus candidate oncornavirus D which includes M-PMV and isolates from a variety of human sources (*1, 20*). Morphologically, it is similar to M-PMV. Its RDDP shows a preference for Mg^{2+} over Mn^{2+}; a property of M-PMV, mouse mammary tumor virus (MMTV), and bromodeoxyuridine-induced guinea pig virus (*13, 21*). Antigenically, it is distinct from M-PMV. The host range is similar to M-PMV and M7 and, as with these viruses, syncytium formation is observed on KC cells (*2, 5, 22*). Lack of growth on SqMLu cells suggests that the isolate is a xenotropic, endogenous squirrel monkey virus. Nucleic acid hybridization studies are required to confirm this. It should be pointed out that M-PMV or other oncornavirus D strains were not knowingly in our laboratory at the time of isolation of the squirrel monkey virus. In addition, this virus has been reisolated from the SqMLu culture on two separate occasions, and viruses with similar properties have been isolated from a lung culture derived from a newborn female squirrel monkey and a variety of tissue cultures from two adult female squirrel monkeys including brain, lung, thymus, kidney, spleen, adrenal, uterus, mammary gland, lymph node, and bone marrow. In no instance was virus detected by polymerase assay or electron microscopy in these squirrel monkey cultures prior to cocultivation with a susceptible cell culture (FCf2Th or MvlLu).

These findings represent the first isolation (to our knowledge) of an oncornavirus with xenotropic properties from a New World monkey. It is of interest that it resembles the oncornavirus D group and not the type C oncornaviruses. This type C group has been observed in the placentas of Old and New World primates by electron microscopy, but only baboon tissues have produced a virus capable of continued growth in culture (*2*). The M-PMV has been isolated from a mammary tumor as well as from normal tissues of the rhesus monkey, where only a portion of the proviral DNA was detected. Therefore, M-PMV does not appear to be a true endogenous virus of the rhesus monkey. There is similar evidence that M-PMV DNA sequences occur in other Old World monkeys, but not in New World monkeys (*23*). It remains to be seen how widespread the squirrel monkey virus is in other New World monkey species, what relationship it has to M-PMV and other candidate oncornavirus D isolates, and whether it has an etiologic role in neoplasia or other disease. We are proposing the designation of squirrel monkey retravirus (SMRV) for this oncornavirus isolate (*24*).

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$(dT)_{12-18}$, and poly(rC) · oligo$(dG)_{12-18}$. Ten microliters of virus (which was concentrated 100-fold by centrifugation and then suspended in 50 m*M* tris-HCl, *p*H 7.3, 10 m*M* dithiothreitol, and bovine serum albumin, 1 mg/ml) were incubated with 10 μl of 50 m*M* tris-HCl, *p*H 7.3, 10 m*M* dithiothreitol, 0.06 percent Triton X-100 for 10 minutes at 2°C. A portion (20 μl) of assay mixture was added, and the resulting 40-μl reaction mixture was incubated for 60 minutes at 37°C. The reaction was stopped by the addition of 0.5 ml of 80 m*M* sodium pyrophosphate, 25 μl of bovine serum albumin (5 mg/ml), and 0.5 ml of 25 percent trichloroacetic acid at 2°C. Acid-insoluble material was collected on glass fiber filters, washed extensively with 10 percent trichloroacetic acid, dried, and dissolved in NCS tissue solubilizer; the radioactivity was then counted in toluene-based scintillation fluid. The reaction mixtures with poly(rA) · oligo$(dT)_{12-18}$ and poly(dA) · oligo$(dT)_{12-18}$ consisted of: 50 m*M* tris-HCl, *p*H 7.3, 70 m*M* KCl, 10 m*M* dithiothreitol, 0.1 m*M* each of deoxyriboadenosine triphosphate (dATP), deoxyriboguanosine triphosphate (dGTP), deoxyribocytidine triphosphate (dCTP), 2.3 μM ^{3}H-labeled thymidine triphosphate (40,000 count/min per picomole), 20 μg of poly(rA) or poly(dA) per milliliter, 20 μg of oligo$(dT)_{12-18}$ per milliliter, and either 5 m*M* $MgCl_2$ or 0.4 m*M* $MnCl_2$. The reaction mixture containing poly(rC) · oligo$(dG)_{12-18}$ consisted of: 50 m*M* tris-HCl, *p*H 7.3, 50 μg of actinomycin D per milliliter, 15 m*M* KCl, 10 m*M* dithiothreitol, 10.7 μM ^{3}H-labeled dGTP (6500 count/min per picomole), 40 μg of poly(rC) per milliliter, 40 μg of oligo$(dG)_{12-18}$ per milliliter, and either 25 m*M* $MgCl_2$ or 0.4 m*M* $MnCl_2$.

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Sodium-Specific Membrane Channels of Frog Skin Are Pores: Current Fluctuations Reveal High Turnover

Abstract. *The reversible sodium transport blocker amiloride causes current fluctuations at the apical membrane of the outer stratum granulosum of frog skin. Their power density spectra reveal that single transport sites translocate more than 10^6 sodium ions per second, which indicates a pore mechanism. The density of open plus amiloride-blocked pores is in the order of 10^8 pores per square centimeter of skin area with 60 millimolar sodium and 18 micromolar amiloride in the outer solution.*

Carrier- and pore-mediated transport through biological membranes can be distinguished by the turnover number of individual transport sites (*1*). While a carrier molecule, which has to move through the lipid phase of the membrane, is not likely to transport more than 10^4 ions or molecules per second, the transport rate of a pore can be several orders of magnitude larger (*2*). Applying this idea to the Na-selective membrane of frog skin, we have attempted to determine the Na turnover of individual transport sites by an evaluation of current fluctuations. The fluctuations were introduced artificially by addition of the drug amiloride, a pyrazine diuretic known to block Na transport reversibly from the outside (*3*). A reversible blocker can be expected to randomly interrupt the Na turnover of individual transport sites. A site will then either conduct fully or, when blocked, not conduct at all. The continuous current *i* passing one site is thus chopped up into small current pulses of varying duration but equal amplitude (*i*). The pulses add up to a mean current which on close inspection will show random fluctuations. The mean current per square centimeter will be

$$I_{Na} = iMP_o \quad (1)$$

where M (cm^{-2}) is the mean density of unblocked plus amiloride-blocked transport sites. The steady-state probability P_o represents the fraction of M not blocked by amiloride and MP_o the mean density of open sites. Statistical evaluation of the fluctuations permits computation of *i* and thus the Na turnover of individual transport sites in the open state.

Isolated abdominal skin of *Rana esculenta* was used at room temperature. It was mounted in a Lucite chamber which left 3 cm^2 exposed to the bathing solutions. The outer solution was K or Na sulfate Ringer solution containing varying concentrations of amiloride. The inner one was K sulfate Ringer solution, which can be expected to depolarize the K-selective inward-facing membranes of the epithelium and to increase their conductance. Thus, transepithelial resistance and potential were largely determined by the apical membrane of the stratum granulosum (*4*). The current component that did not pass the Na-specific channels was determined as the current flowing in the presence of 35 μM amiloride, and was subtracted from the total current to obtain the transcellular Na current, I_{Na}.

Transepithelial voltage was clamped to 0 mv by a voltage clamp circuit with continuous feedback. The input stage of the voltage-sensing amplifier was designed around a matched pair of low-noise transistors (National Semiconductor 2N4250) to minimize feedback current fluctuations arising from this stage. The open-loop clamp gain was 25,000. The short-circuit current was amplified with a gain of 50 μv/na, fed through a high-pass RC-filter with a characteristic frequency of 0.007 hertz, amplified 400 times, and recorded on magnetic tape. Recording periods were 10 to 30 minutes for each amiloride concentration.

The amplified a-c current signal was sampled from the magnetic tape at frequencies of 50 and 5000 hertz by use of an anti-aliasing filter of the Butterworth type (72 db per octave, characteristic frequency set at 80 percent of the maximal analyzed frequency). The digitized signal was divided into 20 records of 4096 words each. A power density spectrum was computed from each record on an IBM 370/58 by use of a fast Fourier transform program. The spectra of 20 records were averaged.

Figure 1A shows five power density spectra in the range 0.3 to 100 hertz. Curves a to d were obtained with a sodium activity of $(Na)_o$ = 60 m*M* in the outer solution and amiloride concentrations of $(A)_o$ = 1.4, 4.3, 12.7, and 35 μM. Spectrum e was obtained with $(Na)_o$ = 0. Spectra a to d are of the Lorentz type expected for exponential relaxation phenomena. They obey the relationship

$$S = S_o/[1 + (f/f_c)^\alpha] \quad (2)$$

where S is the power density (amp^2 sec), S_o the plateau value, f the frequency (hertz), and f_c the corner frequency. The exponent α was found to be in the range 1.75 to 2 (1.8 in Fig. 1A). It is evident