a back-crossed EGFP transgenic strain (B6D2F$_2$ × ICR, F$_6$), they were injected into blastocysts derived from the agouti cross B6D2F$_1$ × ICR.

25. The line EGFP Tg contains a transgene expressing enhanced green fluorescent protein (EGFP) under the control of a CMV-IE enhancer/chicken β-actin promoter combination active in most, if not all, tissues.
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Molecular Analyses of Oral Polio Vaccine Samples

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It has been suggested that the human immunodeficiency virus (HIV), and thus the acquired immunodeficiency syndrome (AIDS) it causes, was inadvertently introduced to humans by the use of an oral polio vaccine (OPV) during a vaccination campaign launched by the Wistar Institute, Philadelphia, PA, USA, in the Belgian Congo in 1958 and 1959. The "OPV/AIDS hypothesis" suggests that the OPV used in this campaign was produced in chimpanzee kidney epithelial cell cultures rather than in monkey kidney cell cultures, as stated by H. Koprowski and co-workers, who produced the OPV. If chimpanzee cells were indeed used, this would lend support to the OPV/AIDS hypothesis, since chimpanzees harbor a simian immunodeficiency virus, widely accepted to be the origin of HIV-1. We analyzed several early OPV pools and found no evidence for the presence of chimpanzee DNA; by contrast, monkey DNA is present.

In the spring of 2000, a committee set up by the Wistar Institute to investigate the "OPV/AIDS hypothesis" (*1, 2*) delivered a total of 14 samples to our laboratory to be analyzed for the presence of chimpanzee DNA (Table 1). The samples were coded by the committee and only after all analyses were concluded and the results transmitted to the committee, were the identity of the samples revealed to us.

Initial experiments showed that DNA could be amplified directly from the OPV samples, whereas extraction procedures resulted in losses of DNA (*3*). In order to estimate how much DNA survived in the 40-year-old vaccine batches, a real-time polymerase chain reaction (PCR) quantitation assay that detects the nuclear *c-myc* gene of mammals was used (*4*). The total amount of DNA in the vaccines as determined by this assay varied from 0 to 7.5 ng/μl (Table 1). For three of the samples, no amplifiable DNA was detected. One of these was a negative control composed of tissue culture medium, one was a late passage of OPV (W Ch 25), and one was an early passage of the OPV (CHAT pool 16 A-5) from the Wistar Institute. All other samples, including CHAT pool 13 that was used in the Congo in the late 1950s, contained detectable DNA. In general, it is noteworthy that no correlation exists between the age of the samples and the amount of DNA retrieved. Thus, the extract of the Sabin I vaccine produced in 2000 contained 5 pg DNA per μl, whereas the CHAT pool 13 produced at the Center for Disease Control (CDC) in 1960 contained 857 pg DNA per μl vaccine.

To identify the source species of the cells used in vaccine production, amplifications of a 151–base pair (bp) portion (with primers) of the mitochondrial (mt) 12*S* rDNA were performed from all 14 samples. The negative control and one late passage of OPV from the Wistar Institute (W Ch 25), where the quantitation failed to detect DNA, yielded no amplification product. All products amplified from the other samples were cloned, and the inserts of 10 clones from each amplification were sequenced. All samples, except CHAT 23 7.7 and the Sabin I vaccine, yielded multiple sequences that differed by up to 20 substitutions within a single amplification. The occurrence of multiple sequences in a single amplification can be a frequent occurrence when mtDNA is amplified. A common reason for this is the amplification of nuclear insertions of mtDNA. Such insertions are numerous and can sometimes dominate amplifications, whereas amplifications of the organellar mtDNA may even be absent, particularly from sources that contain little mtDNA relative to nuclear DNA (*5*). Because intra- or interindividual differences are unlikely in the 12*S* ribosomal gene, which carries little variation within species, we assume that the majority of variation among the clones is due to the amplification of nuclear mtDNA insertions. The multiplicity of mtDNA sequences obtained make the 12*S* rDNA sequences unsuitable for determining the species used for the vaccine production. For example, for CHAT pool 13, seven different sequences were found among 10 sequences determined. Although one of these showed only two differences to mona monkey (*Cercopithecus mona*) while the clone closest to

Table 1. OPV and control samples used in this study. For each sample, the production date (where available), the closest matching species or genera based on the nuclear 28*S* rDNA sequences along with the number of differences, and the DNA concentration (pg/μl sample, averaged over three and two quantitations for the OPV and control samples, respectively) are given. Dashes indicate that no amplification product was seen in two amplification attempts. The OPVs were produced at the Wistar Institute and, where indicated, at the CDC.

Sample number	Nuclear 28S rDNA		DNA (pg/μl)
	Taxon matching	Differences	
	OPV samples		
CHAT 23 7.7	*Macaca*	0	14
WCh-24 57C-40 137-71	*Macaca*	0	53
CHAT Pool 13	*Macaca*	0	344
W Ch 25	–	–	0
CHAT Pool 16 A-5	*Macaca*	0	0
CDC CHAT type I Wy4B-5	*Macaca*	0	47
CDC CHAT 1FL (15 October 1979)	*Homo*	0	7515
CDC CHAT Pool 13 (29 August 1960)	*Macaca*	0	857
Sabin I (20 January 2000)	*Homo*	0	5
	Control samples		
African green monkey	*Chlorocebus*	0	1
Common chimpanzee	*Pan troglodytes*	0	5
Negative control	–	–	0
Macaque	*Macaca*	0	1
Sooty mangabey	*Macaca*	0	6

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chimpanzee carried 11 differences, the fact that the extent of variation among the clones remains unknown makes any statement about their taxonomic affiliation tenuous. Further complicating these analyses is the fact that nuclear insertions may have inserted before the divergence of primate species and may thus be similar or identical in different species, even though their organellar mtDNAs have diverged (*5*–*7*).

To determine more reliably the species used to produce these OPVs, a 141-bp segment (with primers) of the nuclear 28*S* rDNA gene was amplified. All samples except the cell culture control and one OPV sample (W Ch 25) yielded amplification products. These products were cloned, and sequences of 10 clones from each product contained only one DNA sequence per product. In order to compare these DNA sequences to the relevant species, the same DNA segment was determined from five old-world monkeys (*Macaca fascicularis*, *Macaca nemestrina*, *Colobus guereza*, *Chlorocebus aethiops*, *Nasalis larvatus*), one central chimpanzee (*Pan troglodytes troglodytes*), one eastern chimpanze (*P. t. schweinfurthii*), a bonobo (*P. paniscus*), and a human (*8*). The CHAT batch grown in human cells, as well as the Sabin I vaccine, carried a sequence identical to human (Table 1). This sequence differs at one position from bonobo and at two positions from the chimpanzees. All other OPV batches, including CHAT pool 13, yielded one and the same sequence. This sequence is seven nucleotides shorter than the chimpanzee and human sequences and differs at a minimum of 10 positions from the latter species. However, the sequence is identical to *M. fascicularis* and *M. nemestrina* as well as the macaque and mangabey controls obtained by the Wistar Institute from the CDC and included in the original set of 14 unidentified samples.

Because it has been claimed that chimpanzee tissues were cultured at the Wistar Institute at the time of the production of CHAT pool 13 (*2*), it may be argued that the monkey cell cultures used to produce the OPV could have been deliberately or accidentally combined with chimpanzee cells. Such an occurrence would mean that DNA from two species (monkey and chimpanzee) would be present in the OPV samples, with any chimpanzee DNA perhaps being in relatively low abundance. To investigate this possibility, we designed a set of primers that were expected to amplify a 128-bp piece (with primers) of the internal transcribed spacer 1 in the nuclear ribosomal gene cluster of chimpanzees and bonobos, but not from other species. As expected, these primers amplified the chimpanzee and bonobo gene fragments, but failed to amplify the correct fragment from 100 ng of DNA from *Chlorocebus aethiops*, *Nasalis larvatus*, *Colobus guereza*, *Macaca fascicularis*, or humans. By using a dilution series of chimpanzee DNA, it was shown that the primers yielded a visible product from as few as 10 to 50 template DNA copies. Finally, it was tested whether low amounts of chimpanzee DNA could be masked by the presence of monkey DNA. To this end, 50 ng of *M. fascicularis* DNA was mixed with 200, 100, 50, and 5 pg of chimpanzee DNA. In all cases, a strong amplification product of the correct size could be visualized on an agarose gel (*9*). Thus, even in the presence up to a 10,000-fold excess of macaque DNA, chimpanzee DNA was detected by this assay. When these primers were used to amplify from 5 μl of the OPV samples, no amplification products of the correct size could be detected. Assuming that the ape genome contains on the order of 1000 ribosomal genes, this result means that any chimpanzee DNA present in the vaccines must represent less than about one chimpanzee cell per 100 μl of vaccine, or <0.01% of the total DNA present in those samples whose total DNA concentration would allow the detection of such a small DNA component (e.g., CHAT pool 13 and CDC CHAT pool 13).

In conclusion, most of the DNA present in the OPV batches we analyzed is derived from old-world monkeys and not from chimpanzees or bonobos, as previously stated (*10*). It is unlikely that undetected chimpanzee DNA is present, based on the finding that most of the OPV samples, including pool 13 from the Wistar Institute, contain amounts of amplifiable DNA that should make amplifications reproducible. Furthermore, substantial contamination of the tissue cultures used for vaccine production by chimpanzee cells is unlikely, since chimpanzee DNA would have been detected if it constituted >0.01% of the total DNA in the vaccines. Obviously, the samples tested in this study represent only one of maybe four vaccine batches produced by the Wistar Institute and used in the Congo (*11*). However, the results at present give no support for the hypothesis that chimpanzee cells were used to produce the OPV administered in the Congo in 1958 and 1959.

References and Notes

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Virus Maturation Involving Large Subunit Rotations and Local Refolding

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Large-scale conformational changes transform viral precursors into infectious virions. The structure of bacteriophage HK97 capsid, Head-II, was recently solved by crystallography, revealing a catenated cross-linked topology. We have visualized its precursor, Prohead-II, by cryoelectron microscopy and modeled the conformational change by appropriately adapting Head-II. Rigid-body rotations (~40 degrees) cause switching to an entirely different set of interactions; in addition, two motifs undergo refolding. These changes stabilize the capsid by increasing the surface area buried at interfaces and bringing the cross-link–forming residues, initially ~40 angstroms apart, close together. The inner surface of Prohead-II is negatively charged, suggesting that the transition is triggered electrostatically by DNA packaging.

A recurring theme in virus assembly is the initial formation of a precursor particle or procapsid that subsequently transforms into the mature capsid. In general, procapsids and capsids differ markedly in structure. Procapsids were first observed among the double-stranded DNA (dsDNA) bacteriophages (*1*, *2*), but have now also been observed for