Group would induce oxidation of sulfur. Subsequent reduction would produce the $\delta^{34}S$ variations observed in the sulfide minerals.

The interpretation of a significant increase of atmospheric O_2 is consistent with the distribution of red coloration in the Huronian Supergroup. The color is due to hematite and iron hydroxides of nondetrital origin, which are lacking in the three lower groups and occur in the uppermost group (*11, 15*). The $\delta^{34}S$ variation and the occurrence of iron oxides in the Cobalt Group are in accord with an increase in atmospheric O_2 at $\sim 2.2 \times 10^9$ years and its manifestation in the continental sedimentary cycle.

There has been considerable debate concerning the development of O_2 in the earth's atmosphere (*16*). Some arguments have led to the conclusion that the O_2 pressure was fairly high throughout Precambrian time (*17*). In contrast, our conclusion of a significant rise in the atmospheric O_2 content during this time is consistent with the models proposed by Cloud (*18*) and Garrels *et al.* (*19*).

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$$\delta^{34}S = \left[\frac{(^{34}S/^{32}S)_{sample}}{(^{34}S/^{32}S)_{CD}} - 1\right] \times 10^3$$

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High Efficiency DNA-Mediated Transformation of Primate Cells

Abstract. *Tissue culture cells from several mammalian species, including three primate lines, were transfected with recombinant vectors carrying* Escherichia coli *xanthine-guanine phosphoribosyltransferase or Tn5 aminoglycoside phosphotransferase dominant selectable markers. Human HeLa and SV40-transformed xeroderma pigmentosum cells exhibited stable transformation frequencies of at least 10^{-3} (0.1 percent). CV-1, an African green monkey kidney cell line, could be stably transformed with the exceptionally high frequency of 6×10^{-2} (6 percent).*

DNA-mediated introduction of genes into mammalian cells promises to be a powerful method for detecting sequences that control cell growth, confer resistance to toxic drugs, code for surface receptor proteins, or, indeed, alter cell phenotype in any clearly defined way. The importance of this method is well illustrated by the numerous recent publications reporting isolation of cellular oncogenes (*1, 2*). Further use of mammalian gene transfer is limited by the widespread impression that very few cell types are highly competent recipients for DNA-mediated transfection. Mouse NIH/3T3 and Ltk$^-$ cells, which are commonly utilized in genomic DNA transfer experiments, are inappropriate for many applications. We have systematically investigated DNA-mediated stable "transformation" of primate cells with mammalian vectors that carry dominant selectable markers. Our results indicate that primate cells can be equal or superior to murine cells as gene transfer recipients.

We previously described several mammalian vectors that carry the *Escherichia coli* chloramphenicol transacetylase (CAT) gene (*3, 4*). Expression of CAT may be readily assayed in higher eukaryotic cells, and therefore these vectors are useful for optimizing DNA transfection procedures as well as for measuring promoter function. Earlier experiments with CAT vectors indicated that the Rous sarcoma virus long terminal repeat (Rous LTR) directs accumulation of high levels of functional messenger RNA (mRNA) in various mammalian and avian cell types (*4*). This result prompted us to investigate mammalian vectors in which the Rous LTR promoter drives expression of *E. coli* xanthine-guanine phosphoribosyltransferase (gpt) (*5*) or T*n5* aminoglycoside phosphotransferase (neo) (*6*) dominant selectable markers.

The starting construct for these studies was the plasmid pRSVcat (*4*), which is composed of the ampicillin-resistance cistron and the origin of replication from plasmid pBR322 joined to a hybrid eukaryotic transcription unit. The transcription unit in this plasmid is composed of the Rous LTR, CAT coding sequence (cat), and simian virus 40 (SV40) mRNA processing signals, including the small-t intron and early region polyadenylation site. The gpt and neo coding regions, respectively, togeth-

Table 1. Comparison of pSV2neo and pRSVneo stable transformation frequencies. Form I plasmid DNA (5 μg) was added by the calcium phosphate–DNA cotransfection method (*16, 17*) to cells plated on the previous day at 3×10^5 per 25-cm^2 flask. After 48 hours cells were split and replated at the density indicated into medium containing G-418 (800 μg/ml; 39.5 percent active). Colonies (> 50 cells) were counted after 10 to 14 days in selection; transformation frequencies are shown in parentheses.

Recipient cell line	Colonies per 50-cm^2 dish (No.*)	
	pSV2neo	pRSVneo
	10^5 cells plated	
NIH/3T3	25 (2×10^{-4})	91 (9×10^{-4})
CHO	120 (1×10^{-3})	90 (9×10^{-4})
Ltk$^-$	202 (2×10^{-3})	170 (2×10^{-3})
HeLa	29 (3×10^{-4})	215 (2×10^{-3})
	2×10^5 cells plated	
XP_2OS-SV40	22 (1×10^{-4})	150 (8×10^{-4})

*Approximate.

er with mRNA processing signals, were substituted for the corresponding cat/SV40 segment from pRSVcat to generate pRSVgpt and pRSVneo (Fig. 1). An additional plasmid, pRSV-β-globin, was derived from pRSVcat and pSV2-βG (*7*) to provide a vector into which other coding sequences can be conveniently inserted for expression under Rous LTR control.

We first investigated stable transformation of two murine lines (Ltk$^-$ and NIH/3T3), Chinese hamster ovary (CHO), human xeroderma pigmentosum (XP_2OS-SV40), and HeLa cells, with pRSVneo (*8*). The results, including comparison of stable transformation frequencies obtained with pSV2neo, are shown in Table 1. With XP_2OS-SV40, NIH/3T3, and HeLa cells, pRSVneo yielded four to eight times more colonies than pSV2neo; the higher stable transformation frequencies with the pRSVneo vector may reflect our finding that the Rous LTR directs tenfold higher levels of functional CAT mRNA than the SV40 early promoter in NIH/3T3 and HeLa cells (*4*). With CHO and Ltk$^-$ cells, on the other hand, pRSVneo yielded an equal number or slightly fewer colonies than did pSV2neo. Again, the stable transformation results correspond with transient expression data, since the Rous LTR promoter directed slightly less functional CAT mRNA than the SV40 early promoter in CHO cells (*4*). The finding that CHO cells may be stably transformed with neo vectors (in the absence of carrier DNA) at a similar frequency to Ltk$^-$ cells was unexpected.

Table 2. Comparison of pSV2gpt and pRSVgpt transformation frequencies in CV-1 cells. Plasmid DNA was added as in Table 1. After 48 hours cells were split and replated at the density indicated into MX-HAT selective medium (*5*). Transformation efficiencies are shown in parentheses.

Experiment	Colonies per 50-cm^2 dish (No.*)	
	pSV2gpt	pRSVgpt
	10^5 cells plated	
1	800 (8×10^{-3})	6000 (6×10^{-2})
2	500 (5×10^{-3})	5000 (5×10^{-2})
	10^4 cells plated	
2	18 (2×10^{-3})	805 (8×10^{-2})
3	28 (3×10^{-3})	755 (8×10^{-2})

*Approximate.

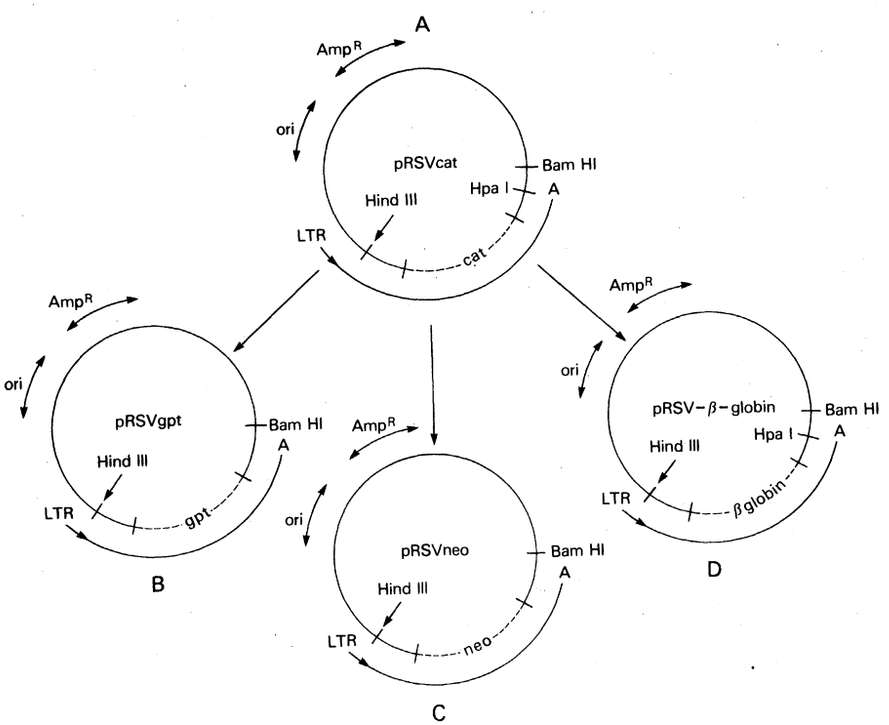

Fig. 1. Construction of RSV vector family. In pRSVcat (A) the Rous sarcoma virus long terminal repeat (*LTR*) functions as a promoter to direct expression of bacterial chloramphenicol acetyltransferase (cat) coding sequences. To construct pRSVgpt (B) a Hind III–Bam HI fragment from pSV2gpt (*5*) containing gpt coding and SV40 mRNA processing signals was inserted in place of the smaller Hind III–Bam HI segment of pRSVcat. To construct pRSVneo (C), a Hind III–Bam HI fragment from pSV2neo (*6*) containing neo coding and SV40 mRNA processing signals was substituted. To construct pRSV-β-globin (D) a Hind III–Hpa I segment from pSV2-βG (*7*) containing rabbit β-globin cDNA and SV40 mRNA processing signals was inserted in place of the smaller Hind III–Hpa I segment of pRSVcat. Unlike the gpt, neo, or cat inserts, the β-globin insert can be directly excised by cleavage at single copy Hind III and Bgl II sites.

Abraham *et al.* (*9*), using the herpes tk marker in the presence of carrier DNA, observed that Ltk$^-$ cells were about 50 times more competent than CHO tk$^-$ cells for stable DNA-mediated transformation.

We next examined stable transformation of monkey kidney CV-1 cells using pSV2gpt and pRSVgpt vectors. In our experience, gpt and neo markers yield similar transformation frequencies with CV-1 cells; because the response of these cells to neo selective conditions is very density-dependent, however, we chose to use the gpt marker. Stable pSV2gpt was observed with an efficiency of 5×10^{-3}, at least 50 times higher than originally reported (*5*). This increased efficiency presumably reflects cumulative effects of improving methods for preparation of plasmid DNA, preparation of calcium phosphate–DNA coprecipitates, and conditions for tissue culture cell growth. We previously used CAT vectors to optimize these parameters with respect to transient expression in CV-1 cells (*3, 10, 11*).

Results of transfecting CV-1 cells with pRSVgpt are also shown in Table 2. As with HeLa and XP_2OS-SV40, the other primate cell types tested, Rous LTR-directed expression of the selectable marker yielded substantially higher stable transformation efficiencies than SV40 early promoter-directed expression: 5 to 8 percent of pRSVgpt-transfected CV-1 cells grown in selective medium formed colonies. Transfection efficiencies were similar whether cells were plated at 200 or 2000 cells per square centimeter, indicating that cross-feeding effects did not give rise to false-positive satellite colonies. Moreover, colonies could be subcloned and grown indefinitely in selective medium, confirming that stable transformation events were being scored (data not shown). Several clones were maintained in the absence of selective medium for six to eight passages, then returned to gpt selection conditions. Inhibition of cell growth was not observed, suggesting, as previously reported (*5*), that stabilization of integrated gpt vector DNA occurs relatively quickly in CV-1 cells.

The frequency of stable transformation that we have obtained with pRSVgpt with CV-1 cells is, surprisingly, at least 25 times greater than with either the gpt or neo markers to transfect Ltk$^-$ and NIH/3T3 cells, and is significantly higher than other investigators have reported with the herpes tk marker to transfect Ltk$^-$ cells (*9, 12*). This extremely efficient transformation appears to be a function both of the high gpt expression

obtained under Rous LTR promoter control and the unusual transformation competence of CV-1 cells. Levels of vector expression immediately after transfection, perhaps reflecting the number of transcriptionally active templates, may determine the probability of a productive stable integration event occurring. Consistent with this hypothesis is the observation that treatment of CV-1 cells with sodium butyrate immediately after transfection results in five- to tenfold increases in both transient expression (assayed with pSV2cat) and stable transformation (assayed with pSV2gpt) (*13*).

The proportion of CV-1 cells that transiently express exogenous vector DNA and then become stably transformed appears to be high. In earlier experiments we used immunofluorescent labeling with CAT antibody to show that, with our standard DNA-mediated transfection conditions, about 10 percent of CV-1 cells transiently express pRSVcat (*4*). Since at least 5 percent of CV-1 cells form colonies after transfection with pRSVgpt under the same conditions, more than half the cells that exhibit transient expression appear to become stably transformed. The number of integrated gpt copies in each of six individual clones examined varied between 1 and 10 (data not shown). No evidence has been found suggesting that either pSV2gpt or pRSVgpt replicates as an episomal plasmid in these cells (*4, 5*).

Although integration of plasmid DNA apparently occurs in the majority of CV-1 cells, our data do not necessarily imply that these cells are exceptionally proficient at integration of exogenous DNA. The overall transformation efficiency may reflect rather the relative stability of integrated copies. Supporting this idea is the finding that neither pSV2gpt$^-$ nor pRSVgpt$^-$ CV-1 colonies appear to segregate at high frequency as do many early mouse L cell herpes tk transformants (*14*).

We did not investigate stable expression of nonselected genes in this study; however, it has been shown that about 25 percent of CV-1 clones isolated after cotransfection with unligated pSV2gpt and pSV2neo vectors express both markers (*6*). This cotransformation frequency, combined with the unusually high competence of CV-1 cells for DNA-mediated transfection, suggests that these cells should find application in screening genomic DNA or eukaryotic complementary DNA (cDNA) expression vector libraries.

Our results as well as our experience with additional cell lines indicate that, for each cell type, it is important both to select a vector with an appropriate promoter and selectable marker combination and to optimize parameters for DNA-mediated transfection (for example, by using the CAT assay). With this approach, it should be possible to stably transform many continuous monolayer cell lines with efficiencies of 10^{-3} (0.1 percent) or higher. Nonimmortalized "primary" cells appear to be less competent recipients for DNA-mediated gene transfer under conditions that we have examined so far. Nevertheless, we find that WI38 human embryo fibroblasts may be stably transformed with pRSVneo at a frequency of at least 10^{-5} (*15*).

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11. Plasmid DNA (form I) was prepared from lysozyme-Triton X100–cleared lysates by two banding steps in CsCl and ethidium chloride equilibrium gradients. Cell lines were grown in Dulbecco's minimal essential medium (DMEM) freshly supplemented with 10 percent fetal calf serum (FCS), glutamine (2 m*M*), penicillin (50 unit/ml), and streptomycin (50 μg/ml). For growth of NIH/3T3 cells, 10 percent calf serum was substituted for FCS; for growth of CHO cells alpha MEM was substituted for DMEM. Cells were split every 4 to 5 days with trypsin (0.25 percent), except CV-1 cells, which required treatment with a solution of trypsin (0.2 percent) and EDTA (2 m*M*). The pCO_2 in tissue culture incubators was periodically adjusted to ensure that medium in the absence of cells equilibrated at *p*H 7.3 to 7.4. Transfection competence of our stock of CV-1 cells frequently declined after ten passages, requiring lower passage cells to be thawed from liquid $nitrogen_2$ storage. Treatment of cells after DNA-mediated transfection with 15 percent (weight to volume) glycerol in Hepes buffer saline ("glycerol shock") was 3 minutes for CV-1 cells but was reduced to as short a time as 30 seconds for other cell types to prevent crenation or shrinkage (or both) of cells before being washed with DMEM. The CAT assay may be used to optimize conditions as follows: transfect 5×10^5 CV-1 cells per 10-cm plate with 10 μg of pSV2cat DNA; harvest 14 to 16 hours after glycerol treatment; assay 10 μl of the cellular extract (which was 100 μl) from one plate in a standard reaction (*3*) containing 0.5 μCi of ^{14}C-labeled chloramphenicol (50 mCi/mmole; New England Nuclear). After a 20-minute incubation period about 20 percent of the ^{14}C-labeled chloramphenicol should be converted to acetylated forms. More detailed protocols describing preparation of DNA, as well as maintenance and transfection of tissue culture cells, are available on request.
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Expression of the Major Neurofilament Subunit in Chicken Erythrocytes

Abstract. *The 70,000-dalton core polypeptide of neurofilaments, thought to exist only in neurons, has been detected in chicken erythrocytes, where it coexists with vimentin and synemin as a component of the intermediate filament network. It is present in the circulating erythroid cells of embryos and young chickens but is nearly absent from the erythroid cells of adults. These findings are inconsistent with current models of intermediate filament expression, but provide another example of unexpected similarities between the nervous and hemopoietic systems.*

Intermediate filaments are linear polymers of polypeptides with subunit molecular masses of 40 to 70 kilodaltons (40 to 70K) and diameters of approximately 10 nm. The filaments can be divided into five general classes, on the basis of subunit biochemistry, antigenicity, and cell type distribution: keratin filaments in most epithelia, neurofilaments in neurons, glial filaments in astrocytes, desmin filaments in muscle, and vimentin filaments in mesenchyme derivatives and in many undifferentiated cells and cultured cells [see (*1*) for a review].

Neurofilaments are composed of a 68 to 70K polypeptide (NF70) (*2*) and two larger polypeptides in association with the core (*3*). This triplet of neurofilament polypeptides (*4*) is now thought to exist only in neurons, although a previous suggestion to the contrary (*5*) resulted from confusion of the 70K neurofilament subunit with the 68K "heat shock" protein, which is found constitutively in