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Selection for animal cells that express the *Escherichia coli* gene coding for xanthine-guanine phosphoribosyltransferase

(pBR322–simian virus 40 DNA vectors/transformation of animal cells/integration of DNA/xanthine phosphoribosyltransferase)

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ABSTRACT **Cultured monkey (TC7) and mouse (3T6) cells synthesize an *Escherichia coli* enzyme, xanthine-guanine phosphoribosyltransferase (XGPRT; 5-phospho-α-D-ribose-1-diphosphate:xanthine phosphoribosyltransferase, EC 2.4.2.22), after transfection with DNA vectors carrying the corresponding bacterial gene, *Ecogpt*. In contrast to mammalian cells, which do not efficiently use xanthine for purine nucleotide synthesis, cells that produce *E. coli* XGPRT can synthesize GMP from xanthine via XMP. After transfection with vector–*Ecogpt* DNAs, surviving cells producing XGPRT can be selectively grown with xanthine as the sole precursor for guanine nucleotide formation in a medium containing inhibitors (aminopterin and mycophenolic acid) that block *de novo* purine nucleotide synthesis. Cells transformed for *Ecogpt* arise with a frequency of 10^{-4} to 10^{-5}; they appear to be genetically stable in as much as there is no discernible decrease in XGPRT formation or loss in their ability to grow in selective medium after propagation in nonselective medium. Although several of the vector–*gpt* DNAs can replicate in monkey and mouse cells, none of the transformants contain autonomously replicating vector–*gpt* DNA. Rather, the *gpt* transformants contain one to five copies of the transfecting DNA associated with, and most probably integrated into, cellular DNA sequences. In several transformants, vector-coded gene products for which there was no selection are also synthesized. This suggests that recombinant DNAs containing *Ecogpt* as a selective marker may be useful for cotransformation of nonselectable genes.**

Considerable progress has been made in developing procedures for the introduction, propagation, and maintenance of selected genes in mammalian cells (1–5). One objective of these studies is to obtain an assay for the function of isolated genes *in vivo*. Such an assay, along with current techniques for gene cloning, *in vitro* mutagenesis, and rapid DNA sequence determination, could uncover additional correlations between the expression and regulation of genes and their structure and chromosomal organization. The availability of general transducing vectors would also permit the construction of novel cellular genomes, a capability that has proved valuable in physiologic and genetic studies of prokaryote organisms.

We have used simian virus 40 (SV40) as a transducing vector; specifically, regions of the SV40 genome have been replaced *in vitro* by selected DNA segments, and the resulting recombinant DNAs have been propagated as viruses with complementing helpers in cultured monkey cells (2, 6). But this experimental design has several shortcomings: (*i*) Only DNA segments smaller than 5 kilobases (kb) can be transduced in this way; (*ii*) the host cell is killed during the course of the infection, precluding the opportunity to monitor the transduced gene's expression in continuously multiplying cells; (*iii*) studies are limited to hosts permissive to SV40, a constraint that excludes many specialized and differentiated animal cells as recipients of the transduced genes.

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To circumvent these disadvantages, we sought to develop transducing vectors that can be introduced into a wide variety of mammalian cells and maintained indefinitely, either as autonomously replicating or as stably integrated genetic elements. Because transfection of mammalian cells with DNA is inefficient (1, 7), the recovery of transformants without a selection is impractical. Therefore, our first goal was to obtain a gene whose expression in the transduced cells would allow them to be grown selectively. That purpose has been achieved by the isolation of a gene from *Escherichia coli* (*Ecogpt*) that encodes xanthine-guanine phosphoribosyltransferase (XGPRT; 5-phospho-α-D-ribose-1-diphosphate:xanthine phosphoribosyltransferase, EC 2.4.2.22), a purine salvage-pathway enzyme.

E. coli XGPRT and the analogous mammalian enzyme, hypoxanthine phosphoribosyltransferase (HPRT; IMP pyrophosphate phosphoribosyltransferase, EC 2.4.2.8), catalyze the conversion of hypoxanthine and guanine to IMP and GMP, respectively; the bacterial enzyme also efficiently converts xanthine to XMP (8), a reaction catalyzed only very poorly by the mammalian enzyme (9). We have previously reported that infection of cultured mammalian cells with recombinant DNAs containing the *Ecogpt* segment induces the synthesis of bacterial XGPRT (5). Moreover, HPRT-negative cell lines transfected with appropriate vectors containing the *Ecogpt* gene synthesize XGPRT and grow selectively in hypoxanthine/aminopterin/thymidine medium (5). This finding suggests that *E. coli* XGPRT can provide the purine salvage function of mammalian HPRT. On that premise, we have devised a procedure that permits the selective recovery of *Ecogpt* transformants from among normal nontransformed cells. The selection relies on the fact that *Ecogpt* transformed cells, but not normal cells, can use xanthine to overcome an inhibition of the *de novo* synthesis of GMP.

METHODS

Cell Culture and Selection for Transformants. African green monkey kidney cells (TC7) and mouse fibroblasts (3T6) were maintained in Dulbecco's modified Eagle's medium containing penicillin, streptomycin, and 5% fetal calf serum. Twenty-four hours after seeding approximately 10^6 cells on 100-mm plates, the cultures were transfected with 10–20 μg of one of the plasmid–*Ecogpt* DNAs according to Parker and Stark's modification (7) of Graham and Van der Eb's procedure (1), except that carrier salmon sperm DNA was omitted.

After 3 days at 37°C in Eagle's medium containing 5% fetal calf serum, the transfected cell monolayers were treated with

Abbreviations: SV40, simian virus 40; kb, kilobase(s); XGPRT, xanthine-guanine phosphoribosyltransferase; GPRT, guanine phosphoribosyltransferase; HPRT, hypoxanthine phosphoribosyltransferase; T and t, SV40 large and small tumor antigens, respectively; APRT, adenine phosphoribosyltransferase; DHFR, dihydrofolate reductase; TK, thymidine kinase.

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trypsin-EDTA, and $\approx 5 \times 10^5$ cells were dispersed on 100-mm plates in Eagle's medium containing 10% dialyzed fetal calf serum/xanthine (250 μg/ml)/hypoxanthine (15 μg/ml) or adenine (25 μg/ml)/L-glutamine (150 μg/ml)/thymidine (10 μg/ml)/aminopterin (2 μg/ml)/mycophenolic acid (25 μg/ml). Mycophenolic acid was prepared in 0.1 M NaOH and neutralized with 0.1 M HCl [Ham's F12 medium (GIBCO) supplemented with the same concentrations of xanthine, aminopterin, and mycophenolic acid has also served successfully for the selection]. Twenty-four hours later, the culture fluid was replaced with fresh medium containing the same supplements; thereafter, it was changed every 3 days. Colonies were readily visible in 7–10 days and isolated with cloning cylinders after 14 days. The number of *Ecogpt* transformants was determined by counting colonies after fixation in methanol and staining with 10% Giemsa; transformation frequency is expressed as the fraction of cells plated that grow under the selective conditions.

Mycophenolic acid alone is sufficient to prevent extensive growth of the cells that have been tested, but in some cell lines the block appears to be delayed and initially incomplete. The addition of aminopterin blocks *de novo* synthesis of all purines and, even in the presence of hypoxanthine or adenine to provide for AMP formation (see Fig. 2), the dependence on xanthine for GMP formation is more exacting. Optimal concentrations of inhibitors and supplements for efficient inhibition and restoration of growth, respectively, should be determined for each cell line used. The optimal lethal concentration of mycophenolic acid differs for different cell lines and can be determined by titration in the presence and absence of guanine.

DNA Extraction and Hybridization Procedures. Plasmid DNAs were isolated from *E. coli* as described (10) and centrifuged twice to equilibrium in cesium chloride/ethidium bromide gradients (11). High and low molecular weight DNAs were obtained from cloned cultures of the transformants as described by Wigler *et al.* (12) and Hirt (13), respectively; the Hirt supernatants were extracted once with phenol/chloroform (1:1) and once with chloroform/isoamyl alcohol (24:1) and then treated with ethanol to precipitate the DNA. Approximately 10 μg of high molecular weight transformed-cell DNA was cleaved with restriction endonuclease, subjected to electrophoresis on agarose gels, transferred to diazobenzyloxymethyl or nitrocellulose paper (14–16), and annealed with ^{32}P nick-translated DNA (17) containing the *Ecogpt* sequence ($0.7–2 \times 10^8$ cpm/μg).

Enzyme and T-Antigen Assays. The preparation and assay of cell extracts for purine phosphoribosyltransferase has been described (5). To measure the formation of SV40 tumor antigens in various transformants, cells were labeled for 1 hr at 37°C with 200 μCi of [^{35}S]methionine (1000 Ci/mmol) in 1 ml of medium containing 4% dialyzed fetal calf serum but lacking methionine. Extracts (6) were treated with hamster anti-SV40 T-antigen serum (Flow Laboratories, Rockville, MD), the immune complexes were subjected to electrophoresis on 10–15% gradient $NaDodSO_4$/acrylamide gels, and the labeled protein bands were visualized by autoradiography.

RESULTS

Transfecting Vectors for *Ecogpt*. A set of plasmid DNA vectors that promote the expression of suitably positioned genes or cDNAs after their introduction into cultured animal cells has been developed (Fig. 1). pSV2, the prototype, contains a cDNA or other coding sequence of interest, downstream (3′-proximal) from the SV40 early transcription promoter (at SV40 ori) and upstream (5′-proximal) from a DNA segment that ensures splicing and polyadenylylation of the ensuing transcript (18). pSV3 and pSV5 are derivatives of pSV2 that contain an intact SV40 or polyoma early region, respectively, inserted at a *Bam*HI restriction site. Each of the vectors contains a DNA segment from

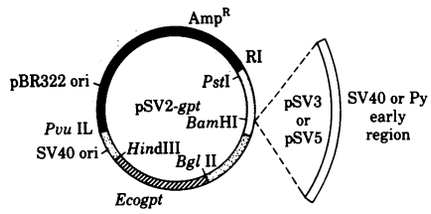

FIG. 1. Structure of pSV-*gpt* vectors. All vectors are shown with the *gpt* segment hatched. The solid black segment represents the 2.3 kb of pBR322 DNA sequences containing the origin of pBR322 DNA replication and the ampicillinase gene. SV40 sequences in pSV2 are shown as lightly stippled regions and include a segment containing the SV40 origin of DNA replication (ori) and the early region promoter (0.71–0.65 map units), the small tumor-antigen intervening sequence (0.56–0.44 map units), and the sequence at which termination and polyadenylylation of SV40 early transcripts (0.19–0.1 map units) occurs. pSV3 and pSV5 DNAs contain, in addition, sequences encoding the early region of SV40 and polyoma, respectively.

the bacterial plasmid pBR322, which enables them to be amplified in *E. coli*. This design permits the transduction of larger DNA segments than can be accommodated with virus genomes as vectors. In addition, the manipulations and expense associated with the construction and propagation of particular recombinant DNAs are greatly reduced. The vectors pSV2, pSV3, and pSV5 containing the cloned segment of *Ecogpt* DNA (ref. 5; see Fig. 1) were used to develop the selection protocol for cells containing *E. coli* XGPRT.

Rationale for Selection of Cells Expressing *E. coli* XGPRT. Purine nucleotides are synthesized *de novo* or by salvage pathways (ref. 19; Fig. 2). In the *de novo* pathway, IMP, the first nucleotide intermediate, is converted to AMP via adenylsuccinate and to GMP via XMP. Salvage of free purines occurs by condensation with phosphoribosyl pyrophosphate: Adenine phosphoribosyltransferase (APRT) accounts for the formation of AMP from adenine, HPRT converts hypoxanthine and guanine to IMP and GMP, respectively. There appears to be *no* mammalian enzyme comparable to the bacterial enzyme for converting xanthine to XMP (9).

Mycophenolic acid, an inhibitor of IMP dehydrogenase (20), prevents the formation of XMP and, therefore, of GMP. The inhibition of purine nucleotide synthesis can be made even more pronounced by the addition of aminopterin, which prevents the *de novo* synthesis of IMP (21). Supplementing the medium with either hypoxanthine or adenine does not reverse the effect of the two inhibitors. However, the inhibition of cell growth by these compounds can be reversed by adding guanine and either hypoxanthine or adenine to the medium, because these bases can be converted to their respective mononucleotides by purine phosphoribosyltransferases. Indeed, the plating efficiency of monkey and mouse cells in medium containing both inhibitors is nearly the same as in normal medium, if adenine and guanine (each at 25 μg/ml) are added. Because normal mammalian cells convert xanthine to XMP very poorly, they cannot grow if medium containing aminopterin and mycophenolic acid is supplemented with adenine and xanthine. However, cells that contain *E. coli* XGPRT grow under these conditions.

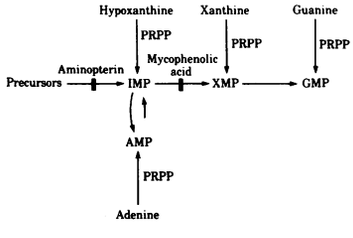

FIG. 2. Pathways of purine nucleotide synthesis and sites of inhibition by aminopterin and mycophenolic acid. Aminopterin blocks *de novo* synthesis of purines from precursors at two steps involving formylation (19). Mycophenolic acid specifically inhibits the conversion of IMP to XMP by IMP dehydrogenase (20). PRPP, phosphoribosyl pyrophosphate.

Transformation of Monkey and Mouse Cells with pSV2-, pSV3-, or pSV5-*gpt* DNAs. About 10^6 monkey (TC7) or mouse (3T6) cells were transfected with 10–20 μg of pSV2, pSV3-, or pSV5-*gpt* DNA; after 3 days in nonselective medium, the cells were treated with trypsin and replated in medium containing aminopterin and mycophenolic acid and supplemented with xanthine and either hypoxanthine or adenine. Cells that received no DNA or pSV2 containing a rabbit β-globin cDNA in place of the *Ecogpt* segment did not survive under the selective conditions; cells transfected with pSV2-, pSV3-, or pSV5-*gpt* DNA yielded 2–25 surviving clones per 10^5 cells plated, transformation being about 5 times more efficient with 3T6.

Several representative cloned TC7 or 3T6 colonies transformed with each of the vectors were subcultured in the selective medium for further study. Selected transformants were assayed for xanthine phosphoribosyltransferase activity by their ability to convert [^{14}C]xanthine to XMP (5). Transformed cell extracts consistently gave 2–8 times higher values than those of comparable extracts from their untransformed progenitors (Table 1). Also, bacterial and mammalian guanine phosphoribosyltransferase (GPRT) activities can be distinguished by the latter's sensitivity to inhibition by hypoxanthine; 20 μM of guanine plus 400 μM of hypoxanthine virtually completely inhibits mammalian GPRT activity, but has little or no effect on that of *E. coli* (5). Thus, GMP formation in the absence of hypoxanthine measures the sum of the cellular and the bacterial GPRT activities whereas measurement in its presence assays the bacterial activity alone. Thus, in extracts of *Ecogpt*-transformed cells, 15–40% of the GPRT activity is not inhibited by hypoxanthine, a property characteristic of the *E. coli* GPRT (Table 2). The electrophoretic mobility of *E. coli* XGPRT differs from that of the mammalian HPRT (5). Clearly, extracts of *Ecogpt*-transformed TC7 and 3T6 cells possess GPRT activities with electrophoretic mobilities characteristic of both the bacterial and the mammalian enzymes (Fig. 3). We conclude that the newly acquired ability of the transformants to convert xanthine to XMP and, thereby, grow in the selective conditions used results from the formation of XPRT by expression of the *Ecogpt* gene.

Stability of *Ecogpt*-Transformed Clones in the Absence of Selection. Several independent *Ecogpt* transformants have been propagated in the absence of mycophenolic acid and aminopterin for about 75 generations. For each line examined so far, after 0, 30, and 75 generations in nonselective media, the ratio of the cells' plating efficiency in selective vs. nonselective media is 1.0 ± 0.2. This suggests that few, if any, of the transformants lose the ability to grow in the selective medium during subculture in the absence of selection for *Ecogpt* expression. Moreover, when extracts of cells grown for 75 generations without selection were assayed for bacterial GPRT activity, the amount was not substantially different from that of cells maintained continuously in the selective medium (data not shown).

Expression of SV40 T Antigens in pSV3-*gpt* Transformants. Of particular interest is whether an unselected genetic marker associated with the vector-*gpt* DNA can be expressed in the transformants. This was examined by testing for the presence of SV40 large and small tumor (T and t, respectively) antigens in representative TC7 and 3T6 cells transformed with pSV3-*gpt* as described in *Methods* (Fig. 4). None of the pSV3-*gpt*-transformed TC7 cells examined so far (four independent isolates) produce T antigen. However, both pSV3-*gpt*-transformed 3T6 cell extracts examined contain proteins that migrate more slowly than SV40 T antigen; whether these proteins are related to T antigen remains to be determined. A band migrating with t antigen is produced in two of the four pSV3-*gpt*-transformed TC7 clones and in both pSV3-*gpt* transformed 3T6 clones (Fig. 4). Thus, in several instances, two linked genetic functions—the *Ecogpt* and an SV40 early gene—appear to be cotransduced.

Physical State and Number of Vector-*gpt* DNA Copies in Transformants. An important question about the transformants is whether *Ecogpt*, and the associated vector DNA sequences, are integrated into the host cells' chromosomal DNA or reside as free, autonomously replicating, plasmid-like elements. We tested the latter possibility by preparing extracts of representative TC7 and 3T6 transformed cells according to Hirt's method (13) and subjecting them to electrophoresis on agarose gels. DNA imprints on diazobenzyloxymethyl (14, 15) or nitrocellulose (16) were hybridized with ^{32}P-labeled *Ecogpt* DNA and autoradiographed. Comparable Hirt extracts from nontransformed TC7 or 3T6 extracts, supplemented with an equivalent of 0.5, 5, or 50 pSV3-*gpt* DNA copies per cell, were analyzed on the same gels for comparison (Fig. 5). There were no de-

Table 1. Detection of *E. coli* xanthine phosphoribosyltransferase activity in extracts of *Ecogpt*-transformants

Cell extract	XMP formed*
TC7	1.1
pSV2/TC7	2.8
pSV3/TC7	4.8
pSV5/TC7	3.6
3T6	0.6
pSV2/3T6	2.0
pSV3/3T6	1.5
pSV5/3T6	4.9

* Expressed as (nmol/min)/mg of protein.

Table 2. Detection of *E. coli* GPRT activity in extracts of transformants in absence and presence of hypoxanthine

Cell extract	GPRT activity		A/B
	Absence (B)	Presence (A)	
TC7	2.0	—	—
TC7/pSV2-*gpt*	1.6	0.31	0.19
TC7/pSV3-*gpt*	2.5	0.42	0.17
TC7/pSV5-*gpt*	2.5	0.34	0.14
3T6	1.0	0.08	—
3T6/pSV2-*gpt*	0.66	0.29	0.36
3T6/pSV3-*gpt*	0.95	0.24	0.17
3T6/pSV5-*gpt*	1.3	0.60	0.38

To calculate A/B, background GPRT activity in nontransformed 3T6 extracts in the presence of hypoxanthine (8% of B) was subtracted from the +GPRT activity of the 3T6 transformants.

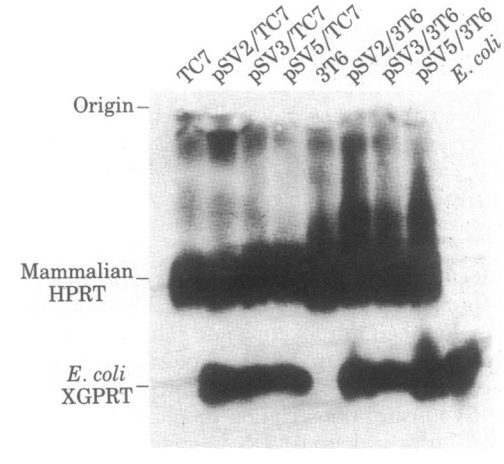

FIG. 3. Detection of GPRT in protein extracts prepared from pSV-*gpt* transformants by *in situ* assay after gel electrophoresis. The electrophoresis of cell extracts and *in situ* assays of GPRT activity have been described (5).

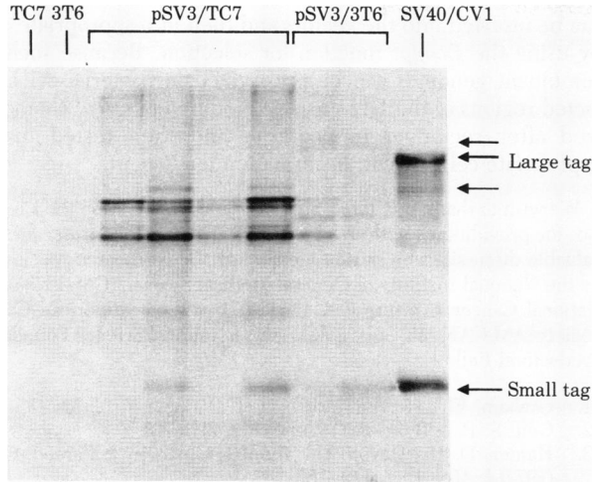

FIG. 4. Immunoprecipitation of SV40 tumor antigens produced in pSV3-*gpt* transformants. Extracts corresponding to 3×10^6 cells were prepared from the transformants and analyzed as described in *Methods*. The first two tracks represent extracts from untransformed TC7 and 3T6 cells and the last from SV40 infected CV1 cells. The other extracts are from independent pSV3-*gpt* transformed TC7 or 3T6 cells.

tectable vector-*gpt* DNA sequences in the Hirt extracts from either pSV3-*gpt*-transformed TC7 cells or pSV5-*gpt*-transformed 3T6 cells. This suggests that vector-*Ecogpt* DNA is most probably not maintained in the transformants as autonomously replicating species resembling the transfecting plasmids.

By contrast, *Ecogpt*-transformed cells contain vector-*Ecogpt* DNA sequences associated with high molecular weight chromosomal DNA. Fig. 6 *left* shows the results of hybridizations of restriction endonuclease-cleaved cell DNAs from various TC7 and 3T6 transformants with a ^{32}P-labeled *Ecogpt* DNA probe. *Sac* I does not cleave pSV2-*gpt* DNA; consequently, with cellular DNA from pSV2-*gpt* transformants, each hybridizing band represents at least one copy of all or part of a transfecting DNA sequence embedded in the cellular DNA. *Eco*RI cleaves both pSV2- and pSV3-*gpt* DNAs only once; therefore, a single, integrated, uninterrupted vector-*gpt* DNA segment should yield two bands, each containing a segment of vector-*gpt* DNA

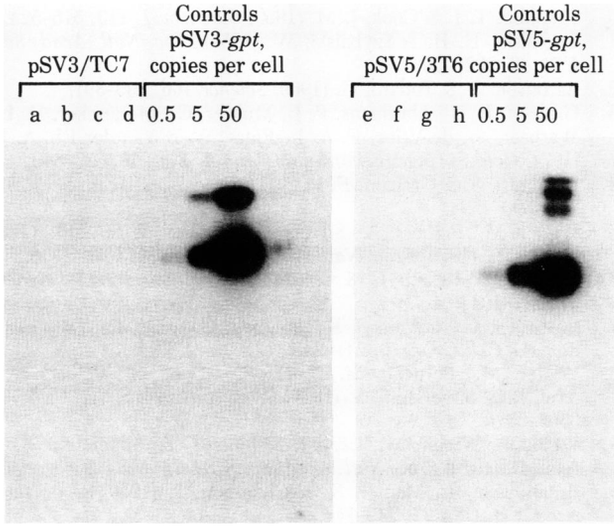

FIG. 5. Analysis of "free" vector DNA in pSV3-*gpt*-transformed TC7 and pSV5-*gpt*-transformed 3T6 cells. Each lane contains Hirt supernatant from 5×10^6 cells. Lanes: a–d, supernatant from pSV3-*gpt*-transformed TC7 cells; e–h, supernatant from pSV5-*gpt*-transformed 3T6 cells. Controls, supernatants from normal cells supplemented with pSV3-*gpt* or pSV5-*gpt* DNAs at 0.5, 5, or 50 equivalents.

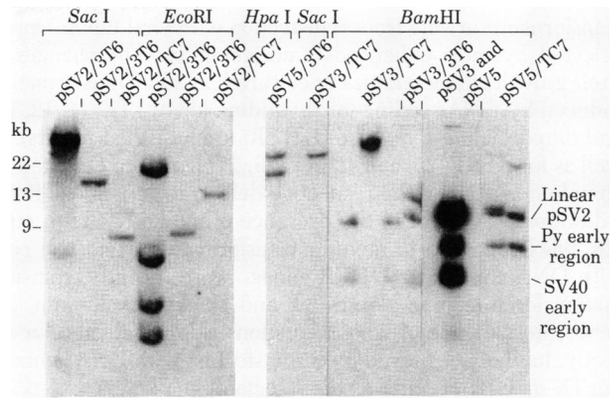

FIG. 6. Vector-*Ecogpt* DNA sequences in the high molecular weight DNA extracted from pSV-*gpt* transformants. (*Left*) Cell DNA from transformants was digested with *Sac* I or *Eco*RI endonucleases, subjected to electrophoresis in 1% agarose and transferred to diazobenzyloxymethylcellulose paper. The imprints were hybridized with nick-translated ^{32}P-labeled pSV2-*gpt* DNA. Size markers were from *Bgl* II endonuclease cleaved phage λ DNA. (*Right*) Cell DNA from pSV3-*gpt* and pSV5-*gpt* transformants was digested with *Sac* I, *Hpa* I, or *Bam*HI and analyzed in *left*, except that transfer was to nitrocellulose paper. The hybridization probe was a mixture of ^{32}P-labeled ^{32}P-labeled pSV3-*gpt* and pSV5-*gpt* DNAs. Size markers were from a mixture of *Bam*HI-cleaved pSV3-*gpt* and pSV5-*gpt* DNAs (≈100 pg of each).

plus flanking cellular DNA sequences. A tandemly repeated or very closely spaced arrangement would yield a band containing full-length vector-*gpt* DNA.

Sac I or *Eco*RI digestion of the DNAs extracted from several pSV2-*gpt* transformants of 3T6 and TC7 cells suggests that the number of vector-*gpt* copies per transformed cell genome is small (in the 1–5 range). Integration of a vector-*gpt* copy near its *Eco*RI restriction site or deletion of that restriction site could account for the occurrence of only one labeled band after digestion of two of the cell DNAs with *Eco*RI.

Conceivably, pSV3-*gpt*-transformed TC7 and pSV5-*gpt*-transformed 3T6 cells might contain a larger number of vector-*gpt* DNA copies per genome because the transfecting DNAs can replicate in these hosts (unpublished). However, the *Sac* I digest of a pSV3 transformant and the *Hpa* I digest of a pSV5 transformant indicate this is not the case (Fig. 6 *right*).

Another question concerns the co-integration of nonselected segments of the transfecting DNA (i.e., those sequences unrelated to *Ecogpt* expression): Are they retained, and, if so, are they intact? We examined this point by digesting cell DNAs from several pSV3-*gpt* and pSV5-*gpt* transformants with *Bam*HI and analyzing the products as described above. Because pSV3-*gpt* and pSV5-*gpt* contain the SV40 and polyoma early regions, respectively, at the *Bam*HI restriction site of pSV2 (Fig. 1), cleavage of pSV3- and pSV5-transformed cell DNAs with *Bam*HI should give a full-length pSV2-*gpt* segment or a full-length virus early-region segment, depending on where the recombination between the vector-*gpt* and the host DNA occurred. This analysis shows that such nonselected segments can indeed be co-integrated in an intact form (Fig. 5 *right*), but additional transformants need to be examined, particularly by molecular cloning of the integrated vector-*gpt* DNA segments, to find the precise organization of the integrated copies and their correspondence to the arrangement in the transfecting DNAs.

DISCUSSION

Stable genetic transformation of mammalian cells after transfection with DNA is a relatively rare event; consequently, the recognition and recovery of rare transformants requires a selection for the appropriate phenotype. For example, oncogenic

transformants arising from transfection with viral DNAs can be selected by their enhanced tumorigenicity or by changes in their growth characteristics in culture (22, 23). Transformation induced by DNAs coding for thymidine kinase (TK) (4, 12, 24) and dihydrofolate reductase (DHFR) (unpublished results), as well as for APRT (25) and HPRT (26, 27) has been detected by complementation of the respective defects in variant cell lines. Additionally, growth in the presence of methotrexate *in vitro* (28) has been used to monitor transformation of normal cells with DNA containing DHFR genes. More recently, transformation for human β-globin (29) and chicken ovalbumin (30) genes, neither one of whose functions can be selected for directly, has been achieved by cotransfection with DNA containing TK or DHFR genes as the selectable marker.

A principal shortcoming of present selection systems is the necessity for specific mutant cell lines as recipients of the transforming DNA. Thus, transformation or cotransformation relying on genes coding for TK, APRT, or HPRT as selective markers requires TK^-, $APRT^-$, or $HPRT^-$ cell lines, respectively, for their detection. The scarcity of such mutants among specialized cell types makes a variety of potentially interesting experiments difficult—e.g., the introduction of globin genes into precursor and mature erythroid cells or of chicken ovalbumin genes into steroid sensitive oviduct cells. Dominant-acting genetic markers—e.g., those that can produce a discernible change in the phenotype of normal cells—offer a way out of this difficulty. The isolation of methotrexate-resistant transformants after transfection of normal cells with DNA from drug-resistant cells (28) exemplifies this approach; however, in this instance, the transformation frequency is low and its utility for cotransformation of other genes is limited. Our experiments suggest that *Ecogpt* provides an alternative dominant selective marker; the expression of this gene confers a novel capability on mammalian cells—efficient utilization of xanthine for GMP formation—and, thereby, a means for its selection in normal cells. Thus far, all of the cell lines tested [monkey (TC7, CV1), mouse (3T6, MEL), human (Lesch–Nyhan), and hamster (CHO)] fail to grow in the selective medium, suggesting that transformation and cotransformation using *Ecogpt* as the selective marker is probably feasible in a wide variety of cells.

Although pSV3- and pSV5-*gpt* DNAs replicate in TC7 and 3T6 cells, respectively (unpublished results), the transformation frequency with the permissive pairings was essentially the same as with the nonreplicating vector pSV2-*gpt* DNA or with a nonpermissive pairing (i.e., pSV3/3T6). Moreover, vector-*gpt* DNAs that do replicate in the transfected hosts do not yield transformants containing free or autonomously replicating vector-*gpt* DNAs. Both observations could be accounted for if the transforming event *per se* is not influenced by the replicability or increased copy number of the vector-*gpt* DNA. However, a more likely possibility is that transformants that have autonomously replicating vector-*gpt* DNA are selected against, perhaps because such autonomous replication competes with the cellular genome for limiting replication functions or because elevated levels of XGPRT and its metabolites disrupt cellular metabolism. A necessity to prevent replication and ensure stable integration of pSV3-*gpt* and pSV5-*gpt* DNAs in their respective permissive hosts would account for the absence of T antigens in these transformants. Formation of SV40 T antigen in several pSV3-*gpt*-transformed 3T6 clones can be explained by the fact that the transfecting pSV3-*gpt* DNA does not replicate in 3T6 mouse cells.

The existence of several restriction sites in pSV2-*gpt* DNA and its derivatives [e.g., *Pst* I, *Eco*RI, and *Bam*HI (see Fig. 1)] provides a way to cotransform a variety of cells with other genes of interest. For example, DNA segments containing the human globin (31), chick ovalbumin (32), or hormone gene (33) families can be inserted into the vectors and then into appropriate cells by using the *Ecogpt* function for selection. Because such recombinant genomes can be propagated in bacterial cells, selected regions of the DNA can be modified *in vitro*, characterized after recloning in bacteria, and then tested in the appropriate cells by cotransformation for *Ecogpt*.

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