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Efficient expression of *Escherichia coli* galactokinase gene in mammalian cells

(pBR322–simian virus 40 fusion plasmid/splice deletion/genetic complementation)

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ABSTRACT The *Escherichia coli* galactokinase gene (*galK*) was inserted into a modified early region transcription unit of simian virus 40 (SV40) contained on a bacterial plasmid. Introduction of this pSVK vector into monkey, mouse, and hamster cell lines by transfection resulted in efficient expression of the bacterial *galK* gene. This expression was shown to be dependent upon fusion of the *galK* gene to the early promoter of SV40 and did not appear to require SV40 splice signals. Moreover, expression in these cells could be obtained either transiently, 24–72 hr after transfection, or continuously, after stable transformation. In particular, pSVK-dependent *galK* expression was obtained in a hamster cell line genetically deficient in galactokinase activity. Expression of the bacterial enzyme was shown to complement the galactosemic defect of these cells, thereby allowing their selective survival and growth on galactose as the only carbon source. The ability to readily assay, select for, and potentially select against *galK* expression from pSVK and its derivatives should prove extremely useful in studying eukaryotic gene regulatory signals.

The universality of the genetic code implies that protein coding sequences should be faithfully translated in heterologous systems. We previously demonstrated that certain prokaryotic mRNAs could be translated at high efficiency in both wheat-germ and reticulocyte cell-free translation systems, provided their 5′ ends were modified with a 7-methylguanosine cap structure (1). Apparently, except for the cap structure, the prokaryotic transcripts contained all the information necessary for their efficient expression within a eukaryotic cellular environment. These results suggested that prokaryotic genes that were properly introduced into eukaryotic cells might be efficiently expressed *in vivo*.

However, for a bacterial gene to be expressed in a eukaryotic cell requires that the gene be fitted with all the regulatory information that normally directs the complex steps of transcription and mRNA maturation of eukaryotic genes and that it be efficiently introduced into the cell nucleus. Recent efforts by several laboratories have focused on the development of recombinant vectors that allow new genetic material to be introduced into eukaryotic cells. Defective simian virus 40 (SV40) or other recombinant viruses that can be propagated in certain permissive cell lines or stably integrated into others have been used successfully (reviewed in ref. 2). Derived from these and of particular interest is a recently developed class of vectors that can be propagated in *Escherichia coli* and then can be used to introduce new genes into mammalian cells. These plasmid vectors have been used to obtain expression of inserted genes (including two bacterial genes) in the absence of any ongoing virus infection (3–5).

In this work, we have inserted the *E. coli* galactokinase gene (*galK*) into one of these plasmid vectors in such a way that transcription of the gene is controlled by the SV40 early promoter. When introduced into cells, the vector directs the efficient synthesis of *E. coli galK* enzyme. Moreover, we show that cells can be transformed to stably express *galK* and that this expression can be used to selectively complement cells that are genetically deficient in their own galactokinase gene function. The potential use of this vector system for studying eukaryotic gene regulation signals will be discussed.

METHODS

Plasmid Construction and DNA Preparation. The design and construction of various recombinant plasmids is described in detail in the text and in the legend to Fig. 1. Digestions with *BAL* 31 (a gift of H. Gray) were carried out as described in 200 mM NaCl buffer (6). DNA from appropriate time points (as judged by restriction endonuclease analysis) was purified by phenol extraction and repeated ethanol precipitation before it was used for ligation. *Hin*dIII linkers (Collaborative Research, Waltham, MA) were activated with phage T4 polynucleotide kinase (P-L Biochemicals, Milwaukee, WI) and ATP (0.2 mM final concentration) as described (7) and used at a linker-to-plasmid ratio of 50:1 (mol/mol). DNAs were ligated overnight at 14°C with T4 DNA ligase (P-L Biochemicals) and then used to transform appropriate bacterial hosts. The desired plasmid constructions were identified by a rapid DNA isolation and screening procedure and then amplified and purified by a cleared lysate technique (8) followed by digestion with DNase-free RNase A (0.5 μg/ml), repeated extraction with phenol/chloroform/isoamyl alcohol (25:24:1 vol/vol), and gel filtration on Sepharose 4B (Pharmacia).

DNA Sequence Analysis. DNA sequences were determined by the chemical methods of Maxam and Gilbert (9), or by partial snake venom phosphodiesterase digestion (10, 11).

Cell Culture. African green monkey CV-1 cells, mouse NIH 3T3 cells, and Chinese hamster V6 and R1610 cells [galactokinase-deficient, *hgpt*$^-$ (hpt 13); *hgpt* is the gene for hypoxanthine (guanine) phosphoribosyltransferase; a kind gift of J.-P. Thirion] were propagated in Dulbecco's modified minimal essential medium supplemented with 5% fetal bovine serum (KC Biological, Lenexa, KA). About 2.5×10^6 cells were transfected with 10 μg (unless specified otherwise) of DNA according to Wigler *et al.* (12). Selections for expression of the *E. coli xgpt* gene [the gene for xanthine (guanine) phosphoribosyltransferase] were carried out as described by Mulligan and Berg (13).

R1610 cells were selected for galactokinase expression by incubation from the third day after transfection in glucose-free modified minimal essential medium supplemented with 10%

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Abbreviations: SV40, simian virus 40; t antigen, small tumor antigen.

dialyzed fetal bovine serum and 1 g of D(+)-galactose per liter (14). Individual clones were isolated as described (15).

Gel Analysis of Galactokinase. Cell extracts were prepared as described (16), usually two days after transfection. Galactokinase enzymes were analyzed on 14% starch gels (Electrostarch, Madison, WI) (17) and detected by fluorography (18).

RESULTS

Design and Construction of the pSVK Vector. The procedures used to insert the *E. coli galK* gene into a vector designed to express protein coding sequences in eukaryotic cells are outlined in Fig. 1. The entire *E. coli galK* gene is carried on the plasmid vector pDS26 (see legend to Fig. 1) and is flanked at its 5′ side by unique *Hin*dIII and *Sma* I sites and at its 3′ side by unique *Hpa* I and *Bam*HI sites. These sites were specifically engineered into the vector so that the *galK* gene could be readily obtained on a variety of DNA fragments and introduced by defined *in vitro* fusions into any genetic system.

Because eukaryotic ribosomes are thought to utilize predominantly the cap-proximal AUG triplet to initiate translation (19, 20), we wanted to ensure that the authentic *galK* initiation codon would be the first AUG on the mRNA produced in mammalian cells. On pDS26, there are three ATG triplets between the *Hin*dIII site and the *galK* translation start codon (21). We therefore shortened the leader region preceding *galK* by first linearizing pDS26 with *Hin*dIII and subsequently digesting the ends with the double strand-specific exonuclease *BAL* 31. *Hin*dIII linker molecules were then inserted into the various deletion sites. Individual shortened plasmids were characterized by restriction and DNA sequence analysis, and some were found to have deleted the three ATG triplets upstream of the *galK* gene. One of these plasmids with deletions had the *Hin*dIII linker positioned 35 base pairs upstream of the *galK* translation start site (pDSΔK, Fig. 1).

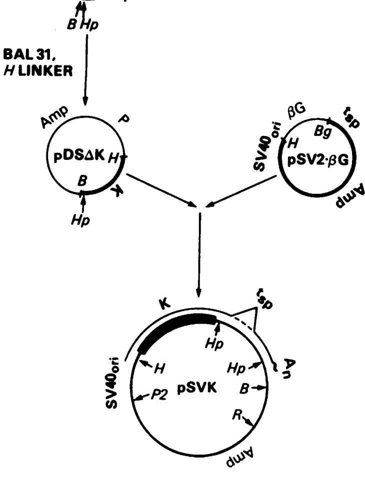

FIG. 1. Construction of the pSVK vector. The starting plasmid pDS26 contains the entire *E. coli galK* gene (K) flanked at its 5′ side by *Hin*dIII (*H*) and *Sma* I (*S*) sites and at its 3′ side by *Hpa* I (*Hp*) and *Bam*HI (*B*) sites. pDS26 is a pBR322 derivative on which the *galK* gene is under the control of a bacterial promoter (P). pDS26 DNA was cut with *Hin*dIII, digested with the double strand-specific exonuclease *BAL* 31, and then recyclized by ligation in the presence of *Hin*dIII linker molecules. One of the resulting plasmids, pDSΔK, was cut with *Hin*dIII and *Bam*HI to produce two fragments, namely the *E. coli galK* gene (shown in bold) and the remainder of the plasmid. Similarly pSV2·βG (5) was cut with *Hin*dIII and *Bgl* II (*Bg*) to produce two fragments, one containing the pSV2 receptor molecule (shown in bold) and the other carrying the rabbit β-globin cDNA (βG). Ligation of the fragments shown in bold yielded the pSVK vector. Restriction sites for those enzymes used to make a particular construction are noted with a cross bar on the inside of the circle, sites for other enzymes are indicated with an arrow on the outside. On the resulting pSVK plasmid, the *E. coli galK* gene is shown as a thick bar. The structure of the presumptive *galK* transcription unit is also indicated. Additional symbols are: Amp, β-lactamase gene conferring ampicillin resistance; $SV40_{ori}$, fragment of SV40 DNA carrying the early promoter and the viral replication origin; t_{sp}, intervening sequence of the SV40 early transcript encoding small tumor antigen (t antigen); A_n, polyadenylylation signal for SV40 early transcripts; *P2*, *Pvu* II restriction site; *R*, *Eco*RI restriction site.

The *galK* coding sequence, carried on pDSΔK, was then introduced into pSV2, a vector that carries certain regulatory signals thought important for gene expression in a eukaryotic background (3, 5). These elements are: (*i*) the SV40 early promoter region, on a fragment carrying the whole viral replication origin, to which the coding sequence can be attached so that its first ATG triplet will also be the first AUG on the transcript; (*ii*) a segment carrying the SV40 t antigen intervening sequence, which is positioned distal to the inserted coding sequence; and (*iii*) the SV40 early transcript polyadenylylation site located downstream from the t antigen splice region. The vector also contains a segment of pBR322 with the bacterial replication origin and the β-lactamase gene conferring ampicillin resistance, thus allowing plasmid constructions and amplification of DNA to be carried out in *E. coli*. pSV2, originally constructed with a rabbit β-globin cDNA insert, was cut with *Hin*dIII and *Bgl* II to remove the β-globin segment and thereby produce its vector form. We then inserted into this vector the *Hin*dIII/*Bam*HI fragment from pDSΔK that carries the *E. coli galK* gene in the proper orientation to form the prototype pSVK plasmid. An important consequence of this cloning scheme is that the arrangement of restriction sites on the pSVK construction allows any of its functional elements to be easily removed or substituted for (Fig. 1). In addition, the vector has unique *Bam*HI, *Eco*RI, and *Pvu* II sites into which additional sequences could be inserted without disturbing the putative *galK* transcription unit.

pSVK Directs *E. coli galK* Expression. We tested if pSVK DNA, brought into mammalian cells by transfection, was capable of expressing the bacterial *galK* gene to produce an enzymatically active product. For these initial experiments, we used the CV-1 African green monkey cell line, whose ability to support transcription from the SV40 early promoter has been well documented (for review see ref. 22). Although CV-1 cells are permissive for SV40 growth, the origin region present on pSVK will not support replication because no SV40 large tumor (T) antigen is available.

CV-1 cells were transfected with pSVK DNA and grown for two days. The *E. coli galK* enzyme can be readily separated from the monkey enzyme by using a starch gel electrophoresis/assay system (ref. 17; Fig. 2*A*, lanes 6 and 1, respectively). This endogenous galactokinase activity provides a useful internal marker for the number of cells analyzed. Extracts from transfected cells were thus analyzed and found to contain a new galactokinase activity (Fig. 2*A*, lane 4). This enzymatic activity was dependent entirely on the presence of the pSVK vector and was lost when the cells were maintained in culture for another week. Identical transfection with other plasmid or phage vectors carrying the *galK* gene but without eukaryotic regulatory sequences did not result in detectable synthesis of this enzyme (Fig. 2*A*, lanes 2 and 3). More importantly, a pSVK derivative from which the entire SV40 fragment upstream of the *galK* gene carrying the SV40 early promoter had been deleted also did not produce any detectable *E. coli galK* enzyme (Fig. 2*B*, lane 9).

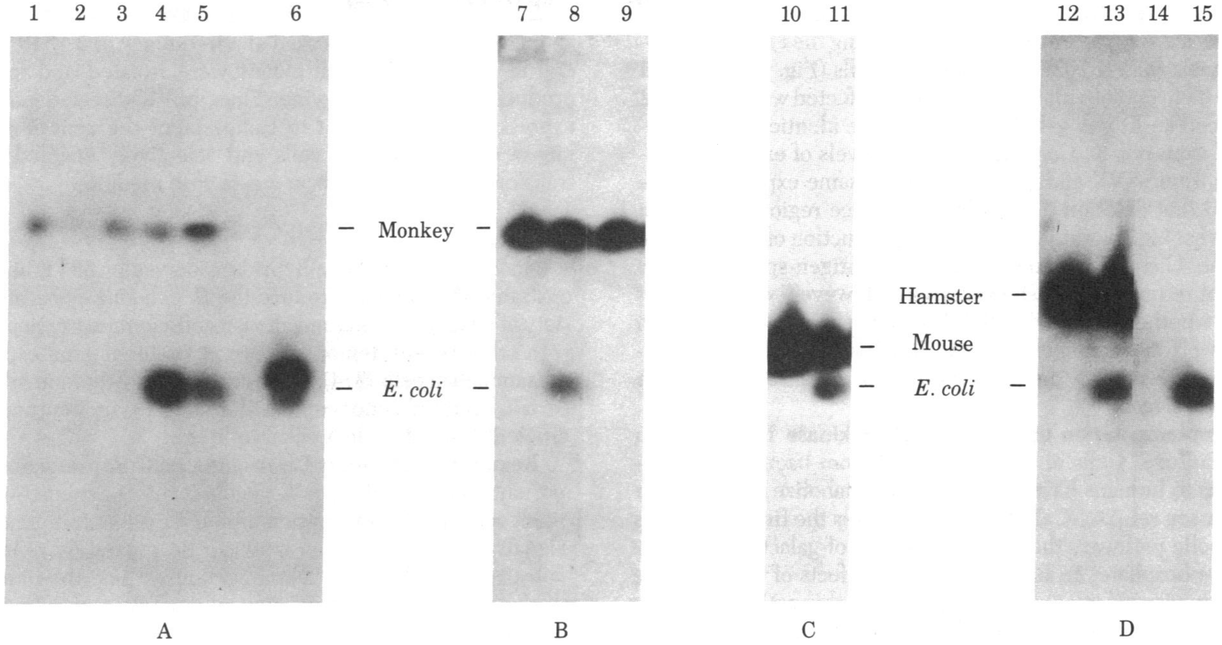

FIG. 2. *E. coli* galactokinase activity in mammalian cells. Fluorographs show the galactokinase activities present in cell extracts subjected to starch gel electrophoresis. The positions of monkey, mouse, hamster, and *E. coli* galactokinase enzymes are indicated. (*A* and *B*) Transfection into CV-1 African green monkey cells. Lanes 1 and 7, transfection with sheared calf thymus DNA; lane 2, pDSΔK DNA; lane 3, DNA of λp*gal*8, a transducing bacteriophage that carries the whole *E. coli gal* operon; lanes 4 and 8, 10 μg of pSVK DNA; lane 5, 2 μg of pSVK DNA; lane 6, extract of *E. coli* C600, *galK*$^-$ cells harboring a *galK*$^+$ plasmid; lane 9, DNA of pSVK101, a pSVK derivative from which the SV40 early promoter/origin region was deleted (see text). (*C*) Transfection into NIH 3T3 mouse cells and selection for the expression of cotransfected *E. coli xgpt* gene. NIH 3T3 cells were transfected with mixtures of DNAs as specified below, incubated for 2 days at 37°C, and then subjected to selection for the dominant marker *E. coli xgpt* (13). Resistant colonies were pooled, grown up, and analyzed on a starch gel. Lane 10, transfection with 10 μg of sheared calf thymus DNA and 5 μg of pSV2-*gpt* DNA; lane 11, 10 μg of pSVK DNA and 5 μg of pSV2-*gpt* DNA. (*D*) Transfection into V6 wild-type Chinese hamster cells and into R1610 *galK*$^-$ mutant cells. Lane 12, V6 cells transfected with sheared calf thymus DNA; lane 13, V6 cells/pSVK DNA; lane 14, R1610 cells/sheared calf thymus DNA; lane 15, R1610 cells/pSVK DNA.

Thus, we have obtained transient expression of the bacterial *galK* gene in monkey cells, and this expression seems to depend on the fusion of the gene to a eukaryotic promoter signal.

By comparing the relative intensities of galactokinase bands produced in pSVK-transfected cells with the ones obtained from assayed bacterial markers we estimate that about 0.1–1.0 unit of enzyme per 10^6 cells is produced (1 unit = 1 nmol/min). If the specific activity of the *E. coli galK* enzyme produced in monkey cells is similar to the one of the purified bacterial enzyme (23), this activity, averaged over all the cells, corresponds to about 2×10^4 to 2×10^5 protein molecules per cell. Presumably, only some fraction of the transfected cells actually expressed the *E. coli galK* enzyme, and expression in individual cells was therefore even more efficient.

To test whether pSVK would also direct the synthesis of *E. coli galK* enzyme in cells that are not permissive for SV40, we used NIH 3T3 mouse fibroblasts. In this case, we cotransfected the cells with pSVK and another pSV2-like vector carrying a dominant selectable marker, the *E. coli xgpt* gene (ref. 3; plasmid pSV2-*gpt*). Beginning 2 days after transfection, the cells were selected for the expression of the *xgpt* gene (13), pooled, and then analyzed for their ability to express both the *xgpt* and the cotransfected *galK* gene. As expected, the resistant population contained *E. coli* xanthine (guanine) phosphoribosyltransferase activity (data not shown). In addition, these cells also contained the bacterial galactokinase activity (Fig. 2*C*, lane 11). This activity was more than 10-fold higher than when assayed 2 days after transfection, prior to the xanthine (guanine) phosphoribosyltransferase selection. Thus, some fraction of the cells that took up, stably integrated, and continuously expressed pSV2-*gpt* did so also for the second, unlinked and unselected, vector, pSVK. This is reminiscent of the results of Wigler *et al.* (12), who demonstrated cointegration of unlinked unselectable genes along with the herpes simplex virus thymidine kinase gene. Detection of pSVK-directed *galK* gene expression in the cotransformed NIH 3T3 cells was possible, despite the nearly identical electrophoretic mobilities of the *E. coli* and mouse galactokinase enzymes (Fig. 2*C*), because the bacterial enzyme produced in mammalian cells always moved somewhat faster in the gels than the one made in *E. coli* (compare lanes 4 and 6 in Fig. 2*A*). The reason for this electrophoretic difference is not known. However, it is likely to result from some posttranslational modification of the protein. We have observed the identical phenomenon when the bacterial *xgpt* gene product, expressed in these same cells from the pSV2-*gpt* vector, was assayed by starch gel electrophoresis (data not shown). This electrophoretic difference was not detected previously by assay on polyacrylamide gels (3).

Thus, both monkey and mouse cells and, as shown below, also hamster cells, can be induced by pSVK transfection to express the bacterial *galK* enzyme. This expression appears to depend on the gene being fused to a eukaryotic promoter signal. Moreover, expression can be obtained either transiently within a few days after transfection or stably by cotransformation with a dominant selectable marker.

t Antigen Splice Is Not Required for *galK* Expression. The SV40 t antigen splice region was originally built into the pSV2 vector because rabbit β-globin cDNA inserted into a similar transcription unit without splicing signals did not lead to the production of cytoplasmic β-globin mRNA (5). We investigated the importance of the SV40 t antigen splicing signals for *E. coli galK* gene expression. The pSVK plasmid was cut with the restriction enzyme *Hpa* I (Fig. 1) and religated to form a derivative that was missing the entire region containing the t antigen splicing signals (pSVK 102). CV-1 monkey cells (Fig. 3) or V6 and R1610 hamster cells (data not shown) transfected with pSVK 102 produced an *E. coli* galactokinase enzyme identical to that obtained from pSVK. Comparison of the levels of expression obtained from pSVK and pSVK 102 in the same experiments indicated that deletion of the t antigen splice region resulted in somewhat higher, rather than lower, production of the bacterial enzyme. Clearly, splicing at the SV40 t antigen-specific signals was not required for *galK* expression. However, we do not yet know whether the pSVK 102-directed *galK* gene expression occurs off an unspliced mRNA. It is possible that other sequences present on the *galK* transcription unit can substitute as splice signals.

Complementation of Genetic Galactokinase Deficiency in Cell Culture. Cells of most organisms from bacteria and protozoans to humans have the ability to metabolize galactose (for review see ref. 24). Galactokinase catalyzes the first step of this metabolic pathway, the phosphorylation of galactose to galactose 1-phosphate. In humans, genetic defects of galactokinase or uridylyltransferase, another enzyme in this pathway, are the causes of the two forms of galactosemia (25, 26). In addition, galactokinase defects have been well characterized in bacteria, certain yeasts, and a Chinese hamster cell line. We have used galactokinase-deficient Chinese hamster cell mutants (14) to study the expression of the bacterial *galK* gene.

Wild-type Chinese hamster cells (V6 cell line) contained a galactokinase activity that was completely absent from extracts of the galactokinase-deficient mutant R1610 cell line (Fig. 2*D*, lanes 12 and 14). When transfected with pSVK DNA, both cell lines produced a new galactokinase band corresponding to *E. coli galK* enzyme (Fig. 2*D*, lanes 13 and 15). In R1610 cells, this pSVK-dependent band was the only detectable galactokinase activity. Thus we have obtained transient expression of the bacterial *galK* gene in hamster cells and in particular in a cell line that is deficient in any endogenous galactokinase activity.

We then tested whether the bacterial galactokinase activity produced in response to pSVK transfection was sufficient to overcome the galactokinase defect of R1610 cells and thereby allow them to grow in the presence of galactose as the only carbon source. Cells were again transfected with pSVK DNA and after 2 days replated in glucose-free medium supplemented with galactose and dialyzed fetal bovine serum. Control cells that had not received pSVK DNA did not survive in this medium. In marked contrast, among pSVK-transfected cells surviving colonies were detected at a frequency of 5×10^{-4}. Several of the resulting cell clones were isolated and found to produce *E. coli galK* enzyme. Thus, pSVK-directed *galK* gene expression was sufficient to complement the genetic galactokinase defect of R1610 cells and selectively enabled pSVK-transformed cells to grow in a galactose medium.

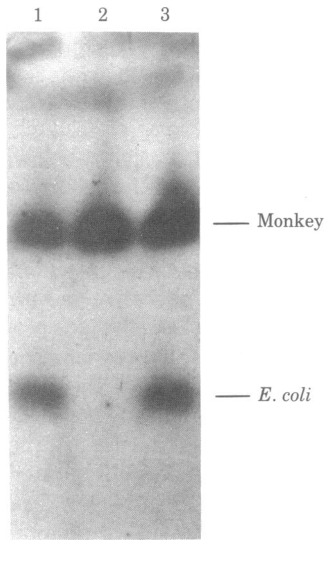

FIG. 3. Galactokinase activity from a pSVK derivative missing the SV40 t antigen splicing signals. The fluorograph of a starch gel electrophoretic galactokinase analysis of monkey CV-1 cell extracts is shown. Lane 1, cells transfected with pSVK DNA; lane 2, sheared calf thymus DNA; lane 3, DNA of pSVK 102, a pSVK derivative from which the *Hpa* I fragment carrying the SV40 t antigen intervening sequence was deleted (see text). The positions of monkey and *E. coli* galactokinases are indicated.

DISCUSSION

We have constructed the hybrid fusion vector pSVK and demonstrated that it will introduce the *E. coli galK* gene into a variety of eukaryotic cells and allow its efficient expression. Along with other recent demonstrations of bacterial gene expression in mammalian cells (3, 4), these results clearly demonstrate that certain bacterial genes contain all the necessary information for efficient translation in a eukaryotic cell.

Regulatory Elements Controlling *galK* Expression. An important feature of the pSVK vector is that each functional element of the *galK* transcription unit (e.g., promoter, gene, splice signals, polyadenylylation site) can be separately removed or substituted with other DNA fragments. Thus, the importance of the regulatory elements controlling *galK* expression can be readily investigated. For instance, we demonstrated that *galK* expression from the pSVK vector is dependent on fusion of the gene to a fragment of SV40 DNA carrying the early SV40 promoter. In addition, we have shown that *galK* expression does not require the t antigen splice region. However, the question whether pSVK 102-directed *galK* expression is mediated by an unspliced mRNA needs to be further investigated. We find the possibility of a substitute splice unlikely for two reasons: (*i*) the observed high level of expression would be surprising if expression depended on splicing and that event were occurring at sites that had not naturally evolved to carry out this function; (*ii*) on pSVK 102, the SV40 early polyadenylylation signal is located only 130 base pairs downstream from the *galK* termination codon (unpublished data). Thus, the putative 5′ and 3′ untranslated sequences are both very short and moreover do not contain any sequences with signficant homology to other splice boundaries (27). Moreover, any splicing that involved *galK* coding sequences would presumably have drastic effects on the size or function of the polypeptide. The pSVK vector also contains the early SV40 polyadenylylation signal, but we do not as yet know whether this signal is required for expression of the bacterial gene.

The modular structure of the pSVK vector should also prove useful in studying sequences from other eukaryotic genes that can fulfill those functions that are required for *galK* gene expression. For instance, it is possible to remove the SV40 promoter region and substitute other eukaryotic DNA fragments that contain analogous regulatory information.

In addition, the vector can be used to study the effects on *galK* expression that result from varying the size and structure of the 5′ untranslated leader region in front of *galK*. For instance, in the *BAL* 31 deletion experiment performed to remove additional ATG triplets 5′ to the *galK* coding sequence (see above and Fig. 1), we obtained a large number of deletion plasmids. These plasmids all contain a novel *Hin*dIII site that is separated from the *galK* coding sequence by various stretches of *E. coli* "leader" sequence. Derived from these, we have constructed a related set of pSVK derivatives that should help elucidate the role and importance of positional effects on the translation efficiency of a particular gene (i.e., *galK*) in mammalian cells.

Gel Assay and Quantitation. Another important element of the pSVK vector system is the ability to readily monitor expression of the bacterial *galK* gene in various cell types despite the presence of an endogenous galactokinase activity. Using this simple and discriminative assay, it is possible to analyze in a variety of cell types precise *in vitro* fusions that recombine various eukaryotic regulatory signals with the *galK* gene present on pSVK. The fact that transient *galK* expression in CV-1 cells was roughly proportional to the amount of pSVK DNA used for transfection (Fig. 2*A*, lanes 4 and 5) suggests that the system might be used to quantitatively assess the function of various gene control signals by monitoring different levels of *galK* expression. However, precise quantitation of transient gene expression after DNA-mediated transfection is potentially affected by a large number of experimental variables. Particularly problematic is the differential gene expression that will result from variations in gene copy number. Recent experiments (unpublished) indicate that these problems can be overcome by introducing into the same vector a second assayable gene (i.e., *E. coli xgpt*) in a similarly constructed transcription unit and using it as an internal standard. Thus, variations in *galK* expression resulting from alterations in its gene control sequences can be accurately measured.

Genetic Complementation and *galK* Selections. We have demonstrated pSVK-dependent expression of the bacterial *galK* gene in a hamster cell line that is genetically deficient in its own galactokinase activity. Moreover, expression of the bacterial enzyme complements the galactosemic defect of these cells, enabling them to survive and grow in a medium containing galactose as the only carbon source. Thus, the galactokinase-deficient hamster cell line allows positive selection for those cells that have been stably transformed by pSVK (or potentially by other pSVK derivatives) and continually express the bacterial *galK* enzyme. Stably transformed cell clones can be studied with respect to the state of the introduced DNA and the precise nature of *galK*-specific transcripts.

In addition to this positive selection for *galK* expression, the system is also amenable to a negative selection (14). 2-Deoxy-D-galactose, an analogue that, when metabolized, blocks sugar chain elongation of glycoproteins (28), could be used to select among the pSVK-transformed hamster cells for those cells that no longer express the *galK* gene. Such positive and negative selections may prove extremely useful in obtaining eukaryotic regulatory site mutations.

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1. Paterson, B. M. & Rosenberg, M. (1979) *Nature (London)* **279**, 692–696.
2. Hamer, D. H. (1980) in *Genetic Engineering*, eds. Setlow, J. K. & Hollaender, A. (Plenum, New York), Vol. 2, pp. 83–101.
3. Mulligan, R. C. & Berg, P. (1980) *Science* **209**, 1422–1427.
4. O'Hare, K., Benoist, C. & Breathnach, R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1527–1531.
5. Howard, B. H., Southern, P., Mulligan, R. C. & Berg, P. (1982) *J. Mol. Appl. Genet.*, in press.
6. Legerski, R. J., Hodnett, J. L. & Gray, H. B. (1978) *Nucleic Acids Res.* **5**, 1445–1464.
7. Lillehaug, J. R. & Kleppe, K. (1975) *Biochemistry* **14**, 1225–1229.
8. Clewell, D. B. & Helinski, D. R. (1969) *Proc. Natl. Acad. Sci. USA* **62**, 1159–1166.
9. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
10. Maniatis, T., Jeffrey, A. & Kleid, D. G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 1184–1188.
11. Brownlee, G. G. & Sanger, F. (1969) *Eur. J. Biochem.* **11**, 395–399.
12. Wigler, M., Sweet, R., Sim, G. K., Wold, B., Pellicer, A., Lacy, E., Maniatis, T., Silverstein, S. & Axel, R. (1979) *Cell* **16**, 777–785.
13. Mulligan, R. C. & Berg, P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2072–2076.
14. Thirion, J.-P., Banville, D. & Noel, H. (1976) *Genetics* **83**, 137–147.
15. Schümperli, D., Loepfe, E., Peterhans, E. & Wyler, R. (1980) *Microbiologica* **3**, 187–203.
16. Peterson, J. L. & McBride, O. W. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1583–1587.
17. Nichols, E. A., Elsevier, S. M. & Ruddle, F. H. (1974) *Cytogenet. Cell Genet.* **13**, 275–278.
18. Laskey, R. A. & Mills, A. D. (1975) *Eur. J. Biochem.* **56**, 335–341.
19. Kozak, M. (1978) *Cell* **15**, 1109–1123.
20. Rosenberg, M. & Paterson, B. M. (1979) *Nature (London)* **279**, 696–701.
21. McKenney, K., Shimatake, H., Court, D., Schmeissner, U., Brady, C. & Rosenberg, M. (1981) in *Gene Amplification and Analysis*, eds. Chirikjian, J. G. & Papas, T. S. (Elsevier/North-Holland, New York), Vol. 2, pp. 383–415.
22. Tooze, J., ed. (1980) *Molecular Biology of Tumor Viruses* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), 2nd Ed., Book B, pp. 125–204.
23. Wilson, D. B. & Hogness, D. S. (1966) *Methods Enzymol.* **8**, 229–240.
24. Segal, S. (1978) in *The Metabolic Basis of Inherited Disease*, eds. Stanbury, J. B., Wyngaarden, J. B. & Fredrickson, D. S., (McGraw-Hill, New York), 4th Ed., pp. 160–181.
25. Gitzelmann, R. (1965) *Lancet* **ii**, 670–671.
26. Mason, H. H. & Turner, M. E. (1935) *Am. J. Dis. Child.* **50**, 359–374.
27. Rogers, J. & Wall, R. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1877–1879.
28. Büchsel, R., Hassels-Vischer, B., Tauber, R. & Reutler, W. (1980) *Eur. J. Biochem.* **111**, 445–453.