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Cloning vectors for expression of cDNA libraries in mammalian cells

(cDNA cloning/expression vector/retroviral transduction/rat thymidine kinase gene)

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ABSTRACT **We have constructed a series of compound cloning vectors (λZD vectors), each consisting of phage λ arms carrying a modified version of the retroviral expression vector pZIP-neoSV(x)1. cDNA, inserted into a cloning site present in the retroviral vector component, is cloned with high efficiency using the λ system. A cDNA library in plasmids is then released by homologous recombination between the retroviral long terminal repeats. Retroviral transduction is achieved by transient expression of the released library in a cell line containing a packaging mutant of Moloney murine leukemia virus, followed by cocultivation of these producers with recipient cells. Transcription of cDNAs in the recipient cells is driven by the strong long terminal repeat promoter, and the transcripts, even from truncated cDNAs, can be expressed because translational start sites have been provided in all three reading frames (tri-initiator). Sequences conferring a recognizable phenotype can be rescued by cell fusion. The functionality of the tri-initiator and the rescue of a rare cDNA have been successfully tested using model systems.**

A general molecular genetic approach to isolate ("rescue") cell-specific transcriptional factors (see ref. 1) can be attempted in two steps. The first step is to generate recipient cell lines by introducing, into cells that do not express a certain gene, recombinant constructs consisting of the gene promoter linked to the sequence of a selectable marker. In a second step, a cDNA library in an expression vector, representing the mRNAs of cells expressing the gene, can be introduced into the recipients. A trans-activating cDNA can then be rescued from the cells that become resistant to the selection medium. Here we present the construction of appropriate expression vectors that are required for the second step of this scheme.

MATERIALS AND METHODS

Vector Constructions. Our constructions (Fig. 1) are derivatives of the λ vector Charon 30 (2) and the retroviral vector pZIP-neoSV(x)1 (ref. 3; henceforth referred to as pZIP, kindly provided by R. Mulligan, B. Roberts, and J. Miller). Specific details about the construction steps will be provided upon request. The two *Eco*RI sites of pZIP were destroyed, and a synthetic duplex oligonucleotide with *Bam*HI-compatible sticky ends,

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5′ GATCTATGGATGGATGGAATTCG 3′
      ATACCTACCTACCTTAAGCCTAG,
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was cloned into the single *Bam*HI site (Fig. 1*b*) in two opposite orientations, generating the plasmids pZD1 and pZD2. The *Bam*HI site was regenerated and a single *Eco*RI cloning site (underlined) was provided. Translational start sites (ATG codons, underlined) in all three reading frames (tri-initiator) were also provided. The transcriptional orientation of the tri-initiator in pZD1 is the same as that of the LTR. Ligation of linearized pZD1 or pZD2 with the *Eco*RI arms of λ Charon 30 yielded the compound vectors λZD31 and λZD32, respectively, in which the orientation of the LTRs is in the direction shown in Fig. 1*b*. The plasmids rpZD1 and rpZD2 (Fig. 1*a*), each containing a single LTR, are released derivatives of λZD1 and λZD2, respectively (see "Plasmid Release", below). For certain experiments (see *Results*) we modified rpZD1 and rpZD2 to generate rpZDD1 and rpZDD2, respectively (see legend to Fig. 1). We also used rpZD1 to derive rpZD5, by replacing a 0.4-kb region (Fig. 1) with a 433-base-pair (bp) fragment containing the polyoma origin of replication and the early gene promoter and enhancer (coordinates 5021 to 162; refs. 4 and 5). Finally, from rpZD5 and λZD31 we derived λZD35 (Fig. 1).

cDNA Library Construction. A cDNA library of rat insulinoma 38 cell (1) mRNA was constructed using the λZD35 vector. After first-strand synthesis, the cDNA was tailed with ≈13 dG residues by use of terminal deoxynucleotidyltransferase (6). Second-strand synthesis was performed by a combination of oligo(dC) priming and "nick-translation" (6, 7) to ensure efficient synthesis and maximal representation of full-length 5′ regions. Following protective methylation and attachment of *Eco*RI linkers (8), 0.3 μg of duplex cDNA was ligated with 6 μg of dephosphorylated vector arms. A library of 18×10^6 recombinants was generated after packaging with Gigapack Gold extracts (Stratagene, La Jolla, CA). A portion of this library (4.6×10^6 recombinants) was amplified and used for plasmid release and transduction (see below). Analysis of 20 randomly picked clones indicated that half of them had inserts with an average size of 1.5 kb.

Plasmid Release. We found that plasmid inserts can be released from the λZD vectors by homologous recombination between the two LTRs, as follows. λZD phages are absorbed at low temperature into *Escherichia coli* strain RRI(pRK248) (9), which is recombination-proficient and contains temperature-sensitive λ repressor molecules. Incoming phages fail to grow lytically but are maintained as large plasmids. If the infected cells are allowed to grow for only a few cell divisions, homologous recombination occurs, followed by segregation of released plasmids and large λ plasmids into different cells. We have calculated in control experiments that there is about one recombination/segregation event releasing a plasmid per 20,000 input phages. Cells with released plasmids are kanamycin-resistant. Cells containing the entire λ genome are lysed by shifting the temperature, thus inactivating the λ repressor.

For plasmid release from the amplified 38 cell cDNA library, we used 3.3×10^{11} plaque-forming units to inoculate a 200-ml overnight culture of RRI(pRK248), grown in NZCYM medium (10) at 30°C. After adsorption for 30 min at

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Abbreviations: LTR, long terminal repeat; Mo-MLV, Moloney murine leukemia virus; SV40, simian virus 40; TK, thymidine kinase.

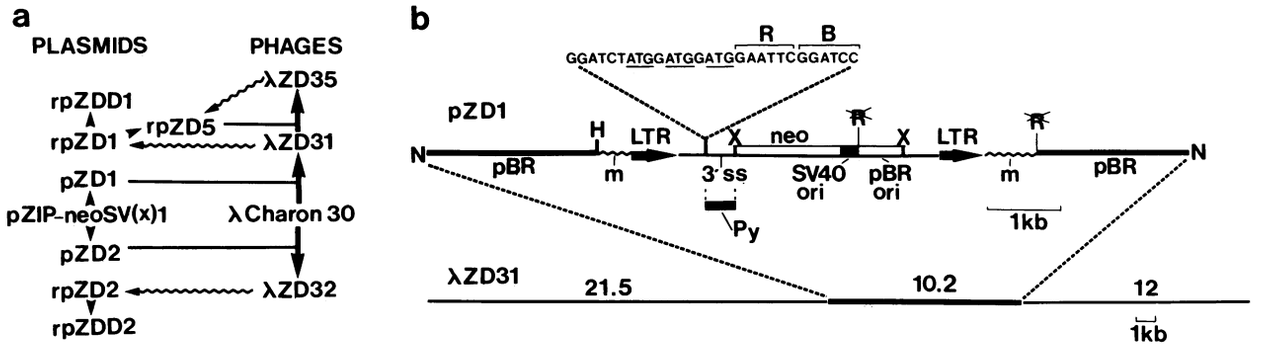

FIG. 1. (*a*) Genealogy of the ZD plasmids and phages and their derivatives (for details see text). Modifications, ligation steps, and plasmid release are indicated by arrowheads, arrows, and wavy arrows, respectively. The rpZDD1 and rpZDD2 derivatives were generated from rpZD1 and rpZD2, respectively, by eliminating the single *Hin*dIII site and then cloning a *Hin*dIII linker into the *Eco*RI site of rpZD1 or into the *Bam*HI site of rpZD2. (*b*) Diagram of the compound vector λZD31, containing the retroviral plasmid vector pZD1, shown in blow-up (dotted lines). The *Eco*RI arms of the λ vector were made blunt and ligated with pZD1 that had been linearized (and filled-in) at the single *Nde* I site. λZD35 was derived by ligating the isolated arms from λZD31 [released after *Xba* I digestion, which cuts once in each long terminal repeat (LTR)] to *Xba* I-linearized rpZD5. The relative arrangement of pBR322 sequences (pBR), mouse genomic sequences (m; wavy lines), the retroviral LTRs (arrows), the G418-resistance gene (*neo*) and the simian virus 40 (SV40) and pBR322 origins (ori) of replication are indicated. The sequence in the region of the tri-initiator and of the single *Eco*RI (R) cloning site is shown in blow-up. Other restriction sites are N, *Nde* I; H, *Hin*dIII; X, *Xho* I; and B, *Bam*HI. These enzymes have additional sites in the compound λ vectors. The positions of two *Eco*RI sites present in the parental pZIP-neoSV(x)1 that have been destroyed (crossed-out) in pZD1 are also shown. The *Nde* I site of pZD1 is not present in λZD31. The position of a 3′ splice site (3′ ss), used for differential splicing (see text), is indicated. The region containing this site has been replaced by polyoma sequence (Py; black bar) in rpZD5 and λZD35. Sizes are in kilobases (kb).

30°C without shaking, 200 ml of 2× YT medium (10) was added, and the culture was shaken for 2.5 hr at 30°C. The cells were collected by centrifugation and resuspended in 400 ml of 2× YT containing 20 mM sodium citrate (addition of citrate prevents reinfection of cells containing released plasmids because it chelates Mg^{2+} required for λ phage propagation; ref. 11). After shaking for 4 hr at 30°C, the culture was diluted into 4 liters of 2× YT (prewarmed at 37°C) containing kanamycin (50 μg/ml) and sodium citrate (20 mM). This final culture was incubated for 24 hr at 42°C in an air-shaker, and then the cells were harvested for plasmid isolation.

Transduction into Rodent Cells. Efficient transduction was achieved by transient expression and cocultivation, a combination of preexisting methods (e.g., refs. 12 and 13), using Wgd5 cells, which are WOP cell derivatives, as producers. WOP cells are mouse 3T3 fibroblasts transformed with an origin-defective polyoma virus (4), which can support replication of constructs like rpZD5. We selected Wgd5 as a gpt^+ clone (resistant to XAT medium; refs. 1 and 14) after cotransfection of WOP cells with pSV2gpt (14) and a packaging mutant of Moloney murine leukemia virus (Mo-MLV) (p3P0). The latter is a derivative of pZIP containing between the LTRs the sequence of the deletion mutant *dl*663-RI, provided by L. Lobel and S. Goff (Columbia University), in which an *Eco*RI linker replaces ≈100 bp between the *Bal* I and *Xma* III sites of Mo-MLV. For transduction, Wgd5 cells were plated at a density of 2×10^6 per plate in a medium that we call XHT (XAT medium without aminopterin or mycophenolic acid; refs. 1 and 14) containing 25 mM Hepes (pH 7.3), and the next day they were treated with mitomycin C (10 μg/ml), a drug of delayed cytotoxicity (cells continue to metabolize for several days, but they never recover to make colonies). A few hours after the drug was washed off, the cells were transfected with calcium phosphate-precipitated DNA (15) of the released library of retroviral plasmids. Following a glycerol shock (16) 4 hr later, the producers were left to recover for about 18 hr. Recipient cells (usually 5×10^5) were added directly onto the plates of the producers, together with Polybrene (8 μg/ml). Recipient cells were replated for selection after 2 days of cocultivation. Using Wgd5 and rpZD5 vector alone (or similar vectors), we obtained in several control experiments between 20,000 and 100,000 G418-resistant colonies of recipient cells per plate of cocultivation.

RESULTS

Features of the Vectors. Because of the *in vitro* packaging procedure, our compound λ vectors can be used for high-efficiency cDNA cloning, allowing representation of rare sequences. The retroviral component provides appropriate transcription signals for expression of the cDNA inserts and can also be exploited for transduction. In the parental retroviral expression vector pZIP (3), the strong Mo-MLV LTR promoter drives the transcription of a cDNA (cloned into the *Bam*HI site) and also of the selectable marker *neo* (individual RNAs are generated by differential splicing; see Fig. 1*b*). Recombinants can be shuttled between mammalian and bacterial cells because the vector contains also the origins of replication of SV40 and pBR322. All of these features are retained in λZD31. In λZD35, the 0.4-kb region of λZD31 (containing a 3′ splice site used for the generation of *neo* by differential splicing) is replaced by a polyoma fragment containing the origin of replication and the early gene promoter and enhancer. Thus, the *neo* gene in λZD35 is driven by the polyoma promoter, whereas cloned cDNA is still expressed from the LTR promoter. Obviation of the need for differential splicing increases the efficiency of transduction of some cDNAs (17). Moreover, the presence of the polyoma origin of replication in λZD35 (and in rpZD5), in addition to the SV40 origin of replication, allows plasmid replication in both WOP (mouse) and COS (monkey) cells. This facilitates both transduction and plasmid rescue not only by COS cell fusion (as with the pZIP system) but also by fusion with WOP cells (see below). Finally, weak translational start sites (tri-initiator), positioned upstream from a unique *Eco*RI cloning site, have been provided in the vectors.

The addition of a tri-initiator to the system is based on the following, experimentally supported, modified scanning model for translational initiation (18, 19). Ribosomes bind to the 5′ end of mRNA and begin scanning (5′ to 3′) without translating until they encounter an AUG initiator. Initiators vary in strength, depending on the context of the sequence in which they are embedded. When a strong initiator is found (conforming to the consensus ACCAUGG), all the ribosomes initiate translation. If an initiator is in a weaker context, some

of the ribosomes begin translation, whereas the rest continue scanning for another AUG. Using as a guide the tabulated sequences of eukaryotic initiators (20) and of known polycistronic messages (18), we designed a tri-initiator with three AUG codons in a weak context, expecting that some ribosomes would initiate translation and some would scan for the next AUG. Thus, full-length cDNAs would synthesize bonafide polypeptides, whereas truncated cDNAs (with a potential to generate functional segments of a protein) could still be translated, since all three reading frames can be utilized.

Functionality of the Tri-Initiator. To examine the ability of the vectors to initiate translation in all three reading frames, we used as a model the hygromycin-resistance gene (*hyg*) from plasmid pHyg (21), provided by B. Sugden. The 5′ end of the *hyg* gene was truncated at specific nucleotides, and the derived fragments were inserted downstream from the tri-initiator in rpZDD1, yielding the series rpZDD1-hyg.1 to -hyg.5 (Table 1). To provide controls (absence of the tri-initiator), we inserted the same fragments in rpZDD2 (rpZDD2-hyg.1 to -hyg.5 series).

The constructs were assayed by transfection into mouse L cells (14). Simultaneous expression and translation of a differentially spliced message from the *neo* gene provided an internal standard for normalization of the results. Table 1 shows the number of resistant colonies on pairs of plates, placed under G418 or hygromycin selection, and the averaged ratio of hygromycin to neomycin resistance. The appearance of hygromycin-resistant colonies indicated that each ATG of the tri-initiator functioned efficiently and at approximately the same level in rpZDD1-hyg.1 to -hyg.3, which lack the authentic *hyg* initiator and in which the first, second, or third ATG is in-frame with the second codon (AAA) of *hyg*. These results were unaffected by the presence of the authentic ATG in rpZDD1-hyg.4 and -hyg.5 (the latter containing an additional upstream out-of-frame ATG of the *hyg* sequence). Of the control constructs rpZDD2-hyg.1 to -hyg.3, only the first (in which the *hyg* coding region is in the same frame as that of the Mo-MLV gene encoding gp80 gag; ref. 22) imparted hygromycin resistance (36% of the efficiency attained with the rpZDD1-hyg derivatives). Thus, the tri-initiator can function as planned; at least 300 times as many resistant colonies were obtained in its presence as in its absence (Table 1).

In rpZDD2-hyg.4, the ATG of *hyg* functioned very efficiently; twice as many hygromycin-resistant colonies were obtained as with any construct of the rpZDD1-hyg series, including the corresponding rpZDD1-hyg.4. To interpret this result, we postulate that in the latter construction some ribosomes interact with the weak out-of-frame AUG codons of the tri-initiator and are diverted into noncoding reading frames. This is consistent with the observed low efficiency of rpZDD2-hyg.5 (<1% of that of rpZDD2-hyg.4); presumably, the strong out-of-frame AUG of *hyg* interferes with the functionality of the authentic initiator, by binding and diverting into the noncoding reading frame the majority of the ribosomes (23). In the corresponding rpZDD1-hyg.5, this interference is canceled; ribosomes initiating at the third AUG of the tri-initiator presumably terminate at a UGA (second upstream triplet from the authentic AUG) and then reinitiate at the authentic AUG of *hyg* (18).

Isolation of the Rat Thymidine Kinase (TK) Gene. To assess the feasibility of our general scheme, we designed a control experiment to rescue a rat TK cDNA, which we expected to be present in low abundance in a rat insulinoma 38 cell library (constructed for the purpose of eventually rescuing insulin gene trans-activators). Twenty plates of Wgd5 cells were transfected with 10 μg of released 38 cell library and 10 μg of carrier DNA, and then cocultivated with TK^- 3T3 cells (provided by M. Wigler). After 2 days the cells were trypsinized, distributed onto 200 plates, and, 1 day later, placed

Table 1. Colony-formation assay to test the functionality of the tri-initiator

	G418	HYG	HYG/G418
ZDD1-HYG.1	1772 (1904,1640)	832 (868,795)	0.47
ZDD1-HYG.2	695 (736,653)	418 (444,392)	0.60
ZDD1-HYG.3	1070 (1091,1049)	716 (724,708)	0.67
ZDD1-HYG.4	713 (787,638)	440 (463,416)	0.62
ZDD1-HYG.5	803 (815, 791)	466 (514,418)	0.58
ZDD2-HYG.1	850 (918,781)	176 (186,166)	0.21
ZDD2-HYG.2	857 (897,817)	2 (3,2,1,1)	0.002
ZDD2-HYG.3	1112 (1166,1057)	1 (1,1,1,0)	0.0008
ZDD2-HYG.4	879 (881,877)	1154 (1163,1145)	1.31
ZDD2-HYG.5	730 (743,716)	8 (10,9,9,5)	0.01
CARRIER ONLY	0 (0,0,0,0)	0 (0,0,0,0)	

The content of each plate of L cells, which had been transfected with one of the indicated constructs, was equally divided into eight plates that were placed under G418 (four plates) or hygromycin (four plates) selection. Thirteen days later, colonies were stained and then counted from two high-density or four low-density plates of each group. For each transfected construct, the numbers of G418- or hygromycin-resistant colonies from each plate (shown in parentheses) and their averages and ratios are shown. An independent experiment (data not shown) yielded similar ratios, although the numbers of resistant colonies were lower. The sequence in the region of the tri-initiator of each construct (verified by DNA sequencing) is shown below.

```
                                  1   2   3
ZDD1-HYG.1  AGATCCCGGATCTATGGATGGATGGAATTCTCAAGCTTTGAAAAAGCCTGAACTC
ZDD1-HYG.2  AGATCCCGGATCTATGGATGGATGGAATTCTCAAGCTTAAAAAGCCTGAACTCAC
ZDD1-HYG.3  AGATCCCGGATCTATGGATGGATGGAATTCTCAAGCTTGAAAAAGCCTGAACTC
ZDD1-HYG.4  AGATCCCGGATCTATGGATGGATGGAATTCTCAAGCTTGATATGAAAAAGCCTG
ZDD1-HYG.5  AGATCCCGGATCTATGGATGGATGGAATTCTCAAGCTTGGCTGCAGGTCGGGGGCAATGAGATATG

ZDD2-HYG.1  AGATCCCGGATCCCCAAGCTTTGAAAAAGCCTGAACTC
ZDD2.HYG.2  AGATCCCGGATCCCCAAGCTTAAAAAGCCTGAACTC
ZDD2-HYG.3  AGATCCCGGATCCCCAAGCTTGAAAAAGCCTGAACTC
ZDD2.HYG.4  AGATCCCGGATCCCCAAGCTTGATATGAAAAAGCCTGAACTC
ZDD2.HYG.5  AGATCCCGGATCCCCAAGCTTGGCTGCAGGTCGGGGGCAATGAGATATG
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The ATG initiator that is presumably responsible for hygromycin resistance in each case is underlined. The second codon of the *hyg* sequence (AAA) is also underlined. A TGA terminator in the sequence of ZDD1-Hyg.5 that is discussed in the text is overlined. The authentic ATG of the *hyg* sequence is boxed, whereas a second, upstream, out-of-frame ATG is underlined with a dotted line.

under hypoxanthine/aminopterin/thymidine (HAT) selection (24). When about 200 HAT-resistant colonies appeared after 10 days, G418 (400 μg/ml) was added to the HAT medium. One week later, only 38 colonies remained on 20 plates. One colony from each of these plates was picked and grown into a culture for analysis (18 of the clones grew successfully). Rescue of proviruses containing rat TK sequence was performed essentially as described (3). Equal numbers of TK^+ transductants and WOP cells were plated together in Dulbecco's modified Eagle's medium with 10% calf serum to generate an almost confluent culture, and the next day the cells were fused (25) using 50% PEG 1000. The medium was changed the next day, and 1 day later the cells were harvested and Hirt supernatants (26) were prepared and used to transform frozen competent *Escherichia coli* DH5α cells (Bethesda Research Laboratories; prepared as described in ref. 27, protocol 3). The identity of the insert in one of the rescued plasmids (pRtk-1) as a rat TK cDNA was first inferred from its restriction map (not shown), which was very similar to that reported for mouse TK cDNA (28), and then verified by partial DNA sequencing (Fig. 2).

Southern blot (29) analysis of genomic DNA from each of the 18 clones, using the insert of pRtk-1 as the hybridization

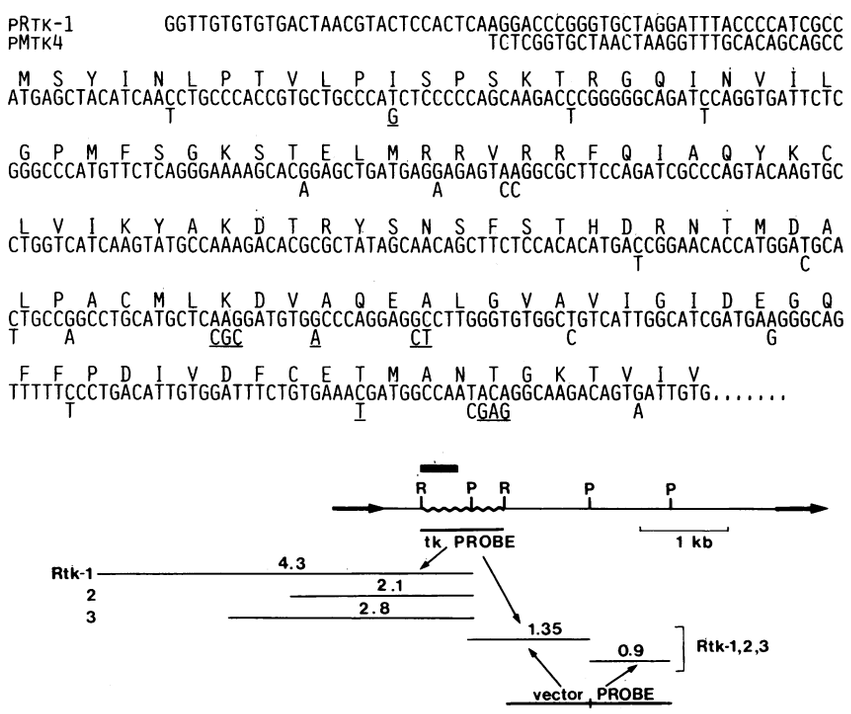

FIG. 2. (*Upper*) Partial nucleotide sequence of the rat TK cDNA insert present in pRtk-1. The sequence begins with the first nucleotide of the 5′ noncoding region present in this clone and is compared with the corresponding sequence present in the mouse cDNA clone pMtk4 (28). The amino acid sequence (one-letter abbreviations of residues) is displayed above the rat TK coding sequence. Nucleotide substitutions in the mouse sequence are shown below the coding sequence, and those leading to amino acid replacements are underlined. (*Lower*) The sequenced region of pRtk-1 (black bar) is indicated in relation to a simplified restriction map showing only *Eco*RI (R) and *Pst* I (P) sites. The fragments used as probes for Southern analysis (Fig. 3) are indicated (the TK cDNA insert or a mixture of two fragments carrying vector sequences). Arrows indicate the fragments (sizes in kb) that each probe detects in the genomic DNA of cellular clones (transductants) Rtk-1, -2, and -3 (see text and Fig. 3).

probe, indicated that 1 of them was a spontaneous revertant, whereas the rest fell into three groups consisting of 12 clones (Rtk-1 group), 2 clones (Rtk-2 group), or 3 clones (Rtk-3 group). Presumably, each group of clones represents a single transduced cell that had divided during cocultivation. The Southern blot profile of one representative from each group (Fig. 3) indicates the presence of two *Pst* I fragments of exogenous rat TK sequence in each transductant (in addition to three apparent *Pst* I fragments of the endogenous mouse gene). In the three transductants, one of the fragments (internal) is of similar, but not identical, size (≈1.35 kb; heterogeneity at the 3′ end of the inserts). The other *Pst* I fragment has a different size in each transductant, as expected; it extends from the *Pst* I site of the cDNA to the most proximal upstream *Pst* I site in the chromosomal flanking sequence (Fig. 2). Thus, we have obtained three independent isolates of rat TK cDNA (as verified by DNA sequencing; see legend to Fig. 3), each occurring in more than one cell line.

When the same membrane (after removal of the TK probe) was rehybridized with vector sequences lying immediately downstream from the cDNA insert, the probe detected (in addition to the 1.35-kb fragment discussed above) an expected 0.9-kb fragment lying between two *Pst* I sites of the *neo* gene (Figs. 2 and 3). The probe also detected additional retroviral insertions in the cell lines (Fig. 3, lanes 1′–3′, arrows). Since control experiments (data not shown) indicated that only about 1% of the recipient cells became G418-resistant, we speculate that recipients in the immediate vicinity of producers can be infected multiple times with recombinant retroviruses, whereas the majority of recipients are not infected at all.

Screening of the initial 38 cDNA library with a pRtk-1 probe yielded 9 positive plaques in 60,000 recombinants. Since in an analogous screening of a mouse cDNA library (27) the number of positive clones was 1 in 100,000, the TK mRNA is indeed not abundant (0.001–0.02%).

DISCUSSION

In addition to their utility for a variety of purposes (see ref. 3 for discussion), our expression vectors can be used for a scheme to rescue trans-activators (see Introduction) if two conditions can be met. (*i*) A single cell- or gene-specific trans-activator, in combination with general transcription factors, must be sufficient for promoter activation. (*ii*) This factor must be the product of a regulatory gene whose expression is cell-specific, and not simply a protein modified in a cell-specific manner (e.g., see refs. 30 and 31). In general, however, our vectors have significant advantages over alternative rescue protocols—for example, the direct use of chromosomal DNA fragments. The latter approach can be easily applied to organisms like *E. coli* and yeast; the wild-type allele of virtually any known mutated gene can be isolated by complementation using a plasmid library (e.g., see ref. 32). On occasion, the same approach can be used with animal cells; several genes have been cloned by transfection of genomic DNA fragments, either by complementation or by bestowing the cells with a new phenotype that somehow can be selected (33–35). However, in addition to the cumbersome methods usually involved in the subsequent identification of the gene of interest, the major disadvantage of this approach, in contrast to the use of expression vectors, is that it is not general; not all mammalian cell lines efficiently take up calcium phosphate-precipitated DNA. Moreover, protocols of transfection or electroporation (36, 37) may not yield adequate levels of a trans-activator mRNA; only low levels of constitutive transcription of the randomly integrated gene in the nonexpressing recipient are expected.

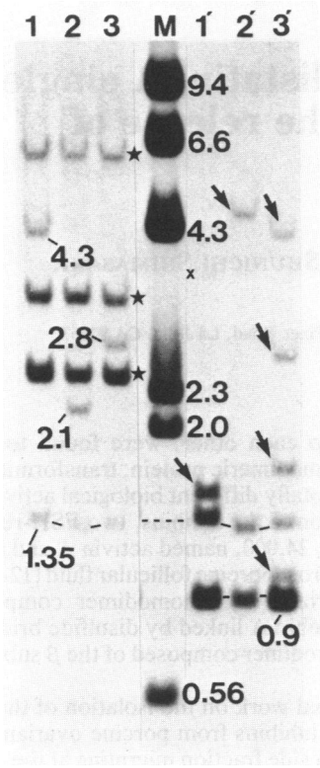

FIG. 3. Southern blot analysis of DNA that was extracted from cultures of the transductants Rtk-1, -2, and -3 (lanes 1–3, respectively). The DNA was digested with *Pst* I, electrophoresed in a 0.8% agarose gel, and then transferred to a nylon membrane. The probes (described in Fig. 2) were TK cDNA sequences (lanes 1–3) or vector sequences (lanes 1'–3'). Stars indicate fragments of the endogenous (mouse) gene. The sizes (in kb) of the fragments carrying TK sequence are shown (see Fig. 2 for interpretation). Arrows indicate fragments of non-TK retroviral recombinants that were independently integrated during transduction. The 0.9-kb fragment detected by the vector probe is common to all integrants (see Fig. 2). This probe also detects an unknown endogenous fragment in all cell lines (faint band denoted by x). The size markers (lane M) are *Hin*dIII fragments of phage λ DNA.

Previously, cDNA expression vectors relying on transient expression in COS cells have been used to isolate rare cDNAs encoding hormones (38, 39) or surface antigens (40). Moreover, a human hypoxanthine phosphoribosyltransferase cDNA was reisolated by stable transformation of L cells with phage λ particles (41). However, such approaches can be successful with only a limited number of cell types. In contrast, the scheme for retroviral transduction that we present is applicable with a variety of differentiated cell types from several mammalian organisms (42, 43). In addition, our vector λZD35 could be used efficiently for λ particle transfection. Finally, because of the presence of the strong Mo-MLV promoter and of both the polyoma and the SV40 origins of replication in our vectors, released libraries could be used successfully for transient expresson assays in either WOP or COS cells.

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