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Bacteriophage Lambda Vector for Transducing a cDNA Clone Library into Mammalian Cells

HIROTO OKAYAMA[1] AND PAUL BERG[2]*

Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, Bethesda, Maryland 20205,[1] and Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305[2]

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We have developed a bacteriophage lambda vector (λNMT) that permits efficient transduction of mammalian cells with a cDNA clone library constructed with the pcD expression vector (H. Okayama and P. Berg, Mol. Cell. Biol. 3:280–289, 1983). The phage vector contains a bacterial gene (*neo*) fused to the simian virus 40 early-region promoter and RNA processing signals, providing a dominant-acting selectable marker for mammalian transformation. The phage DNA can accommodate pcD-cDNA recombinants with cDNA of up to about 9 kilobases without impairing the ability of the phage DNA to be packaged in vitro and propagated in vivo. Transfecting cells with the λNMT-pcD-cDNA recombinant phage yielded G418-resistant clones at high frequency ($\sim 10^{-2}$). Cells that also acquired a particular cDNA segment could be detected among the G418-resistant transformants by a second selection or by a variety of screening protocols. Reconstitution experiments indicated that the vector could transduce 1 in 10^6 cells for a particular phenotype if the corresponding cDNA was present as 1 functional cDNA clone per 10^5 clones in the cDNA library. This expectation was confirmed by obtaining two hypoxanthine-guanine phosphoribosyltransferase (HPRT)-positive transductants after transfecting 10^7 HPRT-deficient mouse L cells with a simian virus 40-transformed human fibroblast cDNA library incorporated into the λNMT phage vector. These transductants contained the human HPRT cDNA sequences and expressed active human HPRT.

The isolation and characterization of cloned genes has been greatly aided by the availability of a wide variety of mutant procaryotic and lower eucaryotic cells and efficient methods for transforming them. This strategy, which relies on complementation of the defect in the cell, has been successful even when the specific function of the gene was unknown and a hybridization probe for the identification of the desired genes was lacking. Several mammalian genes have also been isolated by following this paradigm (11, 14, 15, 22, 25, 26), but the relatively low efficiency of DNA-mediated gene transfer and the scarcity of suitable mammalian cell mutants have hampered this approach. Additional complications arise if the gene contains numerous or very long intervening sequences (e.g., the dihydrofolate reductase [19] and hypoxanthine-guanine phosphoribosyltransferase [HPRT] [11] genes) or if the regulatory region needed for expression is complex (e.g., the regulatory sequences are not closely linked to the promoter).

Since cDNAs represent amplified and already processed versions of genes, we sought a procedure which would permit efficient cloning of full-length cDNAs and would ensure the expression of the cDNAs after transfection into mammalian cells. In that event, phenotypic screening or selection by complementation would permit functional cDNA clones corresponding to genes of interest to be identified. This was achieved by adapting a procedure which yields a high proportion of full-length cDNAs (20) in a plasmid vector that serves both for cloning in *Escherichia coli* and for expression of the cDNA segment in mammalian cells (21). Transcription and processing signals derived from simian virus 40 (SV40) are arranged in the plasmid to ensure transcription, splicing, and polyadenylation of the cloned cDNA segment (Fig. 1). Analogous vectors have been developed which use the adenovirus virus-2 late promoter for mammalian cell hosts (13) or yeast promoters for recovering cDNAs in yeast cells (16, 17).

Our previous experience (21) suggested that if the relevant mRNA species was present at greater than 0.05% of the cellular mRNA, isolation of the desired plasmid-cDNA clones by transduction was feasible. Indeed, mRNA from induced T lymphocytes has yielded cloned expressible cDNAs for a number of lymphokines; in this case, the active clones were detected by their ability to promote lymphokine production in transiently infected COS cells (28; T. Yokota, N. Arai, F. Lee, D. Rennick, T. Masmann, and K. Arai, Proc. Natl. Acad. Sci. U.S.A., in press). However, expressible *hprt* cDNA clones, which were present at a frequency of less than 10^{-5} in a human fibroblast cDNA library, could not be detected after transfection into HPRT-deficient cells by using a hypoxanthine-aminopterin-thymidine (HAT) selection medium (21).

We surmised that the difficulty in detecting such rare cDNA clones was the low efficiency of transformation of the recipient cells and the elimination of potential transformants before they had fully expressed the cDNA-encoded function. To circumvent this shortcoming, we have incorporated the pcD-cDNA library into bacteriophage λ DNA which contains a gene that confers resistance to G418 (*neo*); the recombinant pcD-cDNA-λ*neo* DNA is packaged into phage particles, and these are used to transfect the appropriate recipient cells. After an initial selection for G418 resistance, the *neo*$^+$ transformants are tested for the phenotype corresponding to the desired cDNA. In this paper we describe the λ vector and the sequential selection procedure as it has been applied to the recovery of expressible *hprt* cDNA clones without the use of hybridization probes.

MATERIALS AND METHODS

Cells, bacteria, and enzymes. HPRT-deficient mouse L cells (cell line A9) were maintained in Dulbecco modified

* Corresponding author.

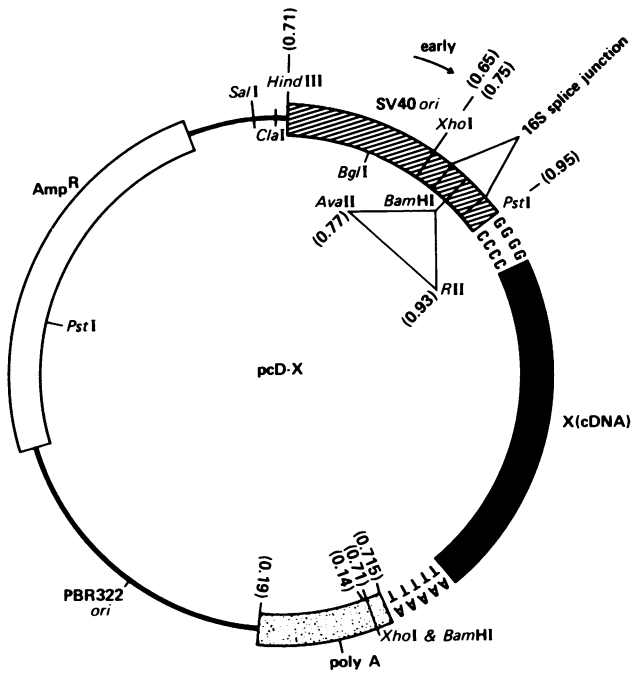

FIG. 1. Structure and component parts of the pcD-cDNA plasmid. The pcD-cDNA plasmid has been described in detail elsewhere (21), but the structure is summarized here to facilitate the presentation in this paper. The principle elements of the pcD vector DNA are a segment containing the SV40 origin of DNA replication (*ori*) and the early-region promoter oriented in the clockwise direction joined to a segment containing the junctions of the 19S and 16S SV40 late-region pre-mRNA intervening sequences (hatched area); the various cDNA segments plus the flanking deoxyguanidylate-deoxycytidilate and deoxyadenylate-deoxythymidylate bridges to the vector produced in the cloning operation, where X symbolizes the particular cDNA insert (solid black area); a fragment containing the SV40 late-region polyadenylation signal (poly A) (stippled area); and the segment containing the pBR322 β-lactamase gene and the origin of replication (pBR322 *ori*) (thin line and open area). The relevant restriction sites referred to in this work are the unique *Sal*I and *Hin*dIII sites of the vector and the *Bam*HI sites within the intron and after the deoxyadenylate-deoxythymidylate bridge. Positions are shown in map units.

Eagle medium containing 10% fetal calf serum in 5% CO_2–95% air at 37°C. Bacteriophage λ762 (27) was obtained from R. Davis (Stanford University), and *Escherichia coli* JC8679 (*thr-1 leuB6 phi-1 lacY1 galK2 ara-14 xyl-5 mtl-1 proA2 his-4 argE3 rpsL31 tsx-33 supE44 recB21 recC22 sbcA23 his-328*) (8) was from R. Weisberg (National Institutes of Health). All restriction enzymes were from New England Biolabs; T4 DNA ligase and synthetic linkers were from Collaborative Research Inc.

Construction of λNMT. The phosphotransferase coding sequence of the *neo* gene (4) in pSV2-*neo* (24) was excised by digestion with *Bam*HI and *Bgl*II endonucleases and inserted into the pcD vector (21) at the *Bam*HI sites. The *Hin*dIII restriction site of the resulting recombinant (pcD**neo*) was eliminated by cleavage with *Hin*dIII endonuclease, filling in the cohesive ends with DNA polymerase I, and religation. A *Sal*I restriction site was also created at the *Tth*I restriction site that occurs in the pBR322 sequence, 150 base pairs downstream from the junction between the plasmid and the SV40 polyadenylation site. Digestion of the resulting modified pcD**neo* with *Sal*I endonuclease released a fragment (1.5 kilobases [kb]) that contained the SV40 early-region promoter, SV40 late 19S mRNA splice junctions, the *neo* coding sequence, and the SV40 late-region polyadenylation site, which could be inserted into the *Xho*I restriction site of λ762 (Fig. 2). The 7-kb bacterial *supF* fragment bounded by *Hin*dIII restriction sites in λ762 was replaced with a 0.5-kb *Hin*dIII fragment obtained from SV40 DNA (map units 0.86 to 0.95).

Construction of λNMT-pcD-*hprt*. pcD-*hprt* (clone praB8) (12) was converted to linear DNA by partial digestion with *Hin*dIII and ligated to *Hin*dIII-digested λNMT DNA. The ligated DNA was transfected into *E. coli* C600 to obtain phage plaques. Recombinant phage containing the *hprt* segment were identified by plaque hybridization with the *hprt* cDNA as probe and by restriction analysis.

Transfer of the cDNA clone library into λNMT. The pcD-cDNA library, prepared from SV40-transformed human fibroblasts (GM637) (21), was recovered from the cloning host, χ1776, by standard Triton X-100–lysozyme extraction followed by two cycles of CsCl equilibrium gradient centrifugation. Approximately 20 μg of the pcD-cDNA was digested with 40 U of *Sal*I at 37°C for 30 min in a reaction mixture (200 μl) containing 150 mM NaCl, 6 mM Tris-hydrochloride (pH 8.0), 6 mM $MgCl_2$, 6 mM 2-mercaptoethanol, and 100 μg of bovine serum albumin per ml. Under these conditions the digestion proceeds to about 50% completion. After phenol-$CHCl_3$ extraction and ethanol precipitation, the digested DNA was dissolved in 50 μl of 50 mM Tris-hydrochloride (pH 9.0) and incubated with 0.05 U of bacterial alkaline phosphatase at 65°C for 20 min. The digestion was terminated by adding 5 μl of 10% sodium dodecyl sulfate, and the DNA was recovered by phenol-$CHCl_3$ extraction followed by ethanol precipitation. The isolated linear pcD-cDNA was inserted into the phage vector as follows. The λNMT phage was amplified in *E. coli* C600 cells and purified by two steps of CsCl block gradient centrifugation (5). The phage DNA was extracted with formamide (5) and digested to completion with *Sal*I. Four micrograms of the *Sal*I-digested λNMT DNA was incubated at 43°C for 1 h in a 14-μl reaction mixture containing 50 mM Tris-hydrochloride (pH 7.5), 6 mM $MgCl_2$, and 8 μg of the bacterial alkaline phosphatase-treated, *Sal*I-digested pcD-cDNA. The mixture was chilled in ice, and 2 μl each 0.1 M dithiothreitol and 10 mM ATP and 1 μl of T4 DNA ligase

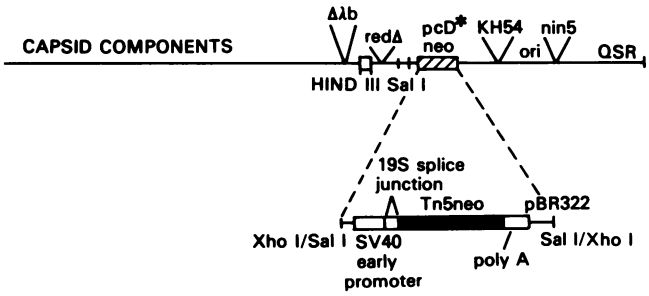

FIG. 2. Genetic and physical structure of λNMT. The genotype of the starting λ bacteriophage was Δ*b*, Δ*red*, KH54, and *nin5*. λNMT contains a mammalian transcription unit that expresses the Tn*5 neo* coding sequence at the *Xho*I restriction site: this segment contains the *neo* gene (shaded area) joined at its 5′ end to a segment containing the SV40 early-region promoter and the SV40 19S late-region pre-mRNA splice junctions and at its 3′ end to the SV40 late-region polyadenylation signal (poly A). The vector also contains a 0.5-kb SV40 *Hin*dIII fragment (map units 0.86 to 0.95) in place of the 7-kb *supF* fragment that resides between the *Hin*dIII restriction sites of λ762.

were added. Ligation proceeded at 16°C overnight, and the ligated DNA was packaged in vitro into phage capsids as described by Enquist and Sternberg (6). The packaged ligated DNA yielded 7×10^6 independent phage plaques, which were then amplified in strain JC8679 on L broth agar plates and stored at 4°C.

Large-scale amplification of the phage cDNA clone library. Strain JC8679 was grown in 24 ml of L broth containing 0.2% maltose at 37°C overnight. Cells were collected by centrifugation, suspended in 12 ml of 10 ml Tris-hydrochloride (pH 7.5) containing 10 mM $MgSO_4$, and infected with 5×10^7 PFU of the stock recombinant phage. After 20 min at room temperature, 100-μl aliquots of the infected-cell suspension were plated on L broth agar plates containing 0.2% glucose, together with 2.5 ml of L broth soft agar (total, 120 plates). The plates were incubated at 37°C for 5.5 h, until the plaques were just about to fuse. The amplified phage were recovered from the lysate by centrifugation and purified by two steps of CsCl block gradient centrifugation.

Transfection of tissue culture cells with λ phage particles. Transfection of A9 cells with recombinant phage was carried out by the method of Ishiura et al. (10), except that HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer (pH 6.9) replaced the BES buffer [*N*,*N*-bis-(2-hydroxyethyl)-2-aminoethane sulfonic acid] and 5×10^{10} PFU were used to transfect 10^6 cells. One day after transfection, the cells were trypsinized and replated at one-fifth the concentration, and 1 day later G418-containing (250 to 400 μg/ml) medium was added to the cells. The G418-containing medium was changed every 2 days for the first week and every 3 days thereafter for a total of 14 to 20 days. The transfected cells were then shifted to a HAT selection medium (3) and incubated at 35°C in 7% CO_2–93% air. The *hprt* transformants were scored or isolated after 2 to 4 weeks in the HAT medium.

Southern transfer analysis of cell DNA. DNA was prepared from cells by the sodium dodecyl sulfate-proteinase K method (1), digested with *Bam*HI, and electrophoresed in 0.8% agarose gels. After transfer of the electrophoresed DNA to nitrocellulose (23), the filter was annealed with the ^{32}P-labeled, nick-translated *Bam*HI-*Pst*I fragment of the *hprt* cDNA (12), and the hybridized bands were visualized by autoradiography.

Isoelectric focusing gel electrophoresis of cell extracts and assay of HPRT in situ. Cell extracts were prepared from exponentially growing cells and electrophoresed on an isoelectric focusing polyacrylamide gel (LKB ampholine PAG plate, pH 5.5 to 8.5) at 1,500 V for 3.5 h under cooling of the gel at 4°C. HPRT on the gel was assayed by the method of Chasin and Urlaub (3).

RESULTS

Construction of λNMT, a phage for transducing mammalian cells. The phage used to construct the transducing vector was λ762 (18), a mutant having deletions in the *b* and *red* regions as well as in the *c*I gene (KH54) and transcription termination site tR2 (*nin5*) (Fig. 2); as a result of these modifications λ762 can only be propagated in a lytic mode. The Tn*5* *neo* gene (4) joined to the SV40 early-region promoter and late 19S mRNA type splice junctions at its 5' end and the SV40 late-region polyadenylation site at its 3' end constitutes an effective mammalian transcription unit that expresses neomycin phosphotransferase and confers resistance to the antibiotic G418 (4, 24). Insertion of the *neo* transcription unit in the *Xho*I restriction site of λ762 yielded λ phage recombinants containing an expressible dominant selection marker for mammalian cells (see below). To accommodate the largest possible insertion into the phage genome, the 7-kb bacterial *supF* fragment, located between two *Hin*dIII restriction sites of λ726, was replaced by a 0.5-kb SV40 *Hin*dIII restriction fragment (map units 0.86 to 0.95). As the λNMT genome is only 38 kb in size, it grows poorly, presumably because the small genome is packaged poorly (6). DNA inserts up to 12 kb can be accommodated in λNMT, and as a result the recombinants grow more efficiently than λNMT. Since the pcD vector is 3 kb in size, pcD-cDNA recombinants with cDNA segments as large as 9 kb can be inserted into the phage DNA and still be propagated as phage. Digestion of λNMT with *Hin*dIII or *Sal*I provides cohesive termini for joining to a similarly cleaved pcD cDNA library.

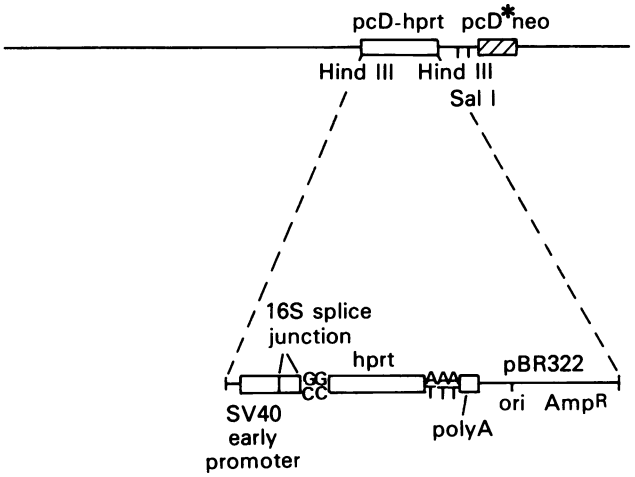

FIG. 3. Structure of λNMT-pcD*hprt*. Plasmid pcD*hprt* (12) was digested with *Hin*dIII to produce linear DNA, and these were inserted into λNMT, replacing the 0.5-kb SV40 fragment. poly A, Polyadenylation site.

Transfection of mammalian cells with λNMT-pcD-cDNA. To verify that λNMT transduces mouse L cells to G418 resistance, suitable cell cultures were transfected with the phage (10^{10} λNMT phage per 10^6 L cells) as described above, and *neo*$^+$ transformants were selected in medium containing 250 to 400 μg of G418 per ml. The frequency of G418-resistant colonies ranged between 0.5 and 1%.

To determine whether sequential selection could be used to isolate selectable markers in the cDNA library, we constructed λNMT-pcD*hprt* (Fig. 3), a recombinant phage with an insert of pcD*hprt* (12). Plasmid pcD*hprt* is a recombinant isolated from an SV40-transformed human fibroblast cDNA library which expresses human HPRT and transforms HPRT$^-$ mammalian cells for growth in HAT medium (3). Transfection of A9 cells (HPRT$^-$) with λNMT-pcD*hprt* phage (10^{10} phage per 10^6 cells) yields HAT-resistant colonies at frequencies between 10^{-3} and 10^{-4}. Sequential selection for the two markers was tested with mixtures of λNMT-pcD*hprt* and λNMT-*supF* (which lacks the pcD*hprt* segment but contains the 7-kb *supF* fragment instead of the shorter SV40 fragment of λNMT). Each phage was grown separately and purified, and the two were mixed so that the ratio of λNMT-pcD*hprt* to λNMT-*supF* was 10^{-3}, 10^{-4}, or 10^{-5}. Approximately 5×10^{10} PFU of each phage mixture was transfected into 10^6 HPRT$^-$ A9 cells as calcium phosphate coprecipitates (see above). G418-resistant colonies were obtained in each case at a frequency approximating that obtained with λNMT alone (about 10^{-2}). Notably, there

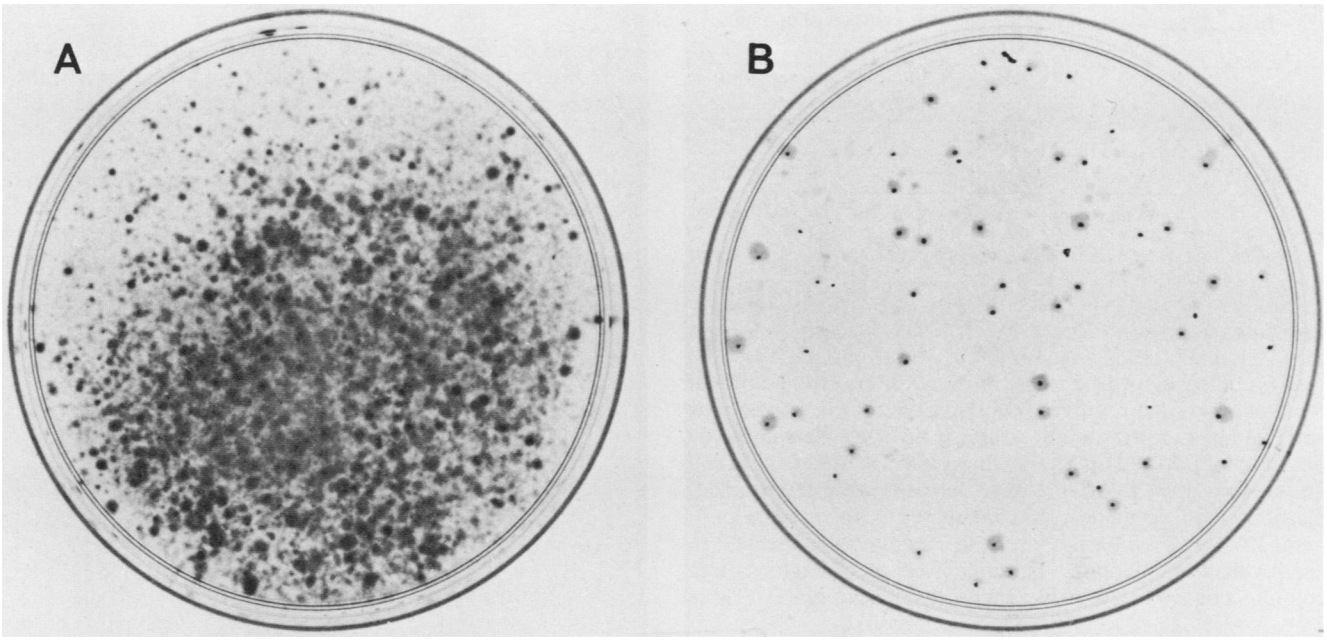

FIG. 4. Sequential selection for G418-resistant, HPRT$^+$ transformants. (A) G418-resistant colonies obtained after transfection with a mixture of bacteriophage containing 1 λNMT-pcD*hprt* clone in 1,000 λNMT-*supF* transformants and growth in a medium containing G418. (B) Colonies surviving after 14 days in the G418-containing medium and then grown in HAT selection medium.

were no HAT-resistant colonies (HPRT$^+$) produced by transfection with any of the phage mixtures, although, as mentioned above, transfection with λNMT-pcD*hprt* alone yielded a high frequency of transformation to HPRT$^+$. However, if the transfected cells were selected first for G418 resistance and then switched to a HAT medium, HPRT$^+$ transformants were obtained at frequencies roughly proportional to the amount of λNMT-pcD*hprt* present in the three mixtures (Fig. 4 and Table 1). These findings suggest that relatively high expression of HPRT, as occurs after transfection with either pcD*hprt* or λNMT-pcD*hprt* alone, is needed for the transformants to survive through the early stages of HAT selection. More specifically, it seems that when λNMT-pcD*hprt* is present at less than 0.1% of the phage mixture, HPRT expression is insufficient to permit cells to survive direct selection in HAT medium, but if the transfectants are first selected for G418 resistance, even a mixture with 0.001% λNMT-pcD*hprt* yields HPRT$^+$ transformants. Presumably, once integrated, the pcD*hprt* transcription unit(s) produces sufficient HPRT to support growth in HAT medium.

These reconstitution experiments indicate that transfections with the phage vector can detect a particular cDNA even if only 1 functional cDNA segment in 10^5 is present in the phage population.

Identification of a functional *hprt* cDNA clone by transduction with a cDNA library of λNMT-pcD-cDNA. Based on the findings described above, cDNA clones expressing the HPRT$^+$ phenotype should be detected even if they occur at a frequency of 10^{-5} in the cDNA library by examining at least 10^6 cells. Practically, it is feasible to transfect $\sim 10^7$ cells (10 to 20 plates), and therefore we expect to detect several expressible *hprt* cDNA clones in a pcD-cDNA library containing about 10^6 independent cDNA clones.

A test of this expectation was to isolate expressible pcD*hprt* clones that were known to be present in the SV40-transformed human fibroblast cDNA clone library (21) by selection alone. The plasmid DNA containing the complete cDNA library (pcD-cDNA) was isolated from *E. coli*, partially digested with *Sal*I, and ligated to *Sal*I-cleaved λNMT DNA (Fig. 5). Packaging the λ DNA in vitro (6) yielded a preparation which produced 5×10^6 phage plaques, nearly 80% of which contained an insert of pcD-cDNA.

TABLE 1. Transfection of A9 cells with λNMT-pcD*hprt* and λNMT-*supF* mixed at various ratios

Phage ratio (λNMT-pcD*hprt*-λNMT-*supF*)[a]	No. of HAT-resistant colonies/10^6 cells
0:1,000	0
1:1,000	110
1:10,000	8
1:100,000	1

[a] The total number of the mixed phage used for transfection was 5×10^{10} PFU per 10^6 A9 cells.

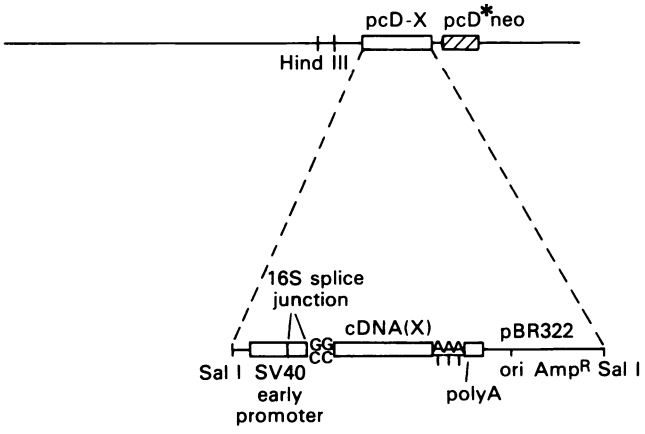

FIG. 5. General structure of λNMT-pcD-cDNA. X symbolizes the cDNA inserts. The pcD-X plasmid DNA can be inserted into the λ DNA in either orientation.

TABLE 2. Transfection of A9 cells with λNMT containing a human pcD-cDNA clone library[a]

cDNA clone library	No. of colonies obtained in selective medium:	
	G418	HAT
pcDGM637	100,000[b]	3

[a] The total number of phage and cells used were 5×10^{11} PFU and 10^7 cells, respectively.
[b] Estimated from the number of colonies counted under a microscope in several visual fields.

Inserting sequences at the *Sal*I restriction site reduced expression of the λ *gam* function which inhibits the host *recBC* nuclease, an antagonist of the λ rolling circle mode of replication. To circumvent this problem, the stock was amplified in a *recBC* mutant strain, JC8679 (8). The amplified phage were purified and transfected into 10^7 A9 cells spread on 10 large dishes. After 1 day, the cells were trypsinized, distributed to 50 dishes, and incubated with medium containing G418. Two weeks later the medium was changed to HAT selection medium. Three to four weeks later, three colonies continued to grow, each on separate plates (Table 2).

To determine whether these colonies survived in HAT medium because they were transformed by phage carrying an insert of pcD*hprt*, DNA from the putative transformants was examined first for the presence of the human *hprt* cDNA sequence (Fig. 6). Cell DNA from each transformant was cleaved with *Bam*HI, electrophoresed, and transferred to nitrocellulose (23), and the blots were hybridized with ^{32}P-labeled, nick-translated human *hprt* cDNA (12). DNA from two of the three HPRT$^+$ transformants yielded *Bam*HI fragments which hybridized with the human cDNA probe (Fig. 6); moreover, the size of each of the fragments was very close to the size of the *Bam*HI fragment produced from the human *hprt* cDNA clone. Judging from the intensity of the bands obtained with each transformed-cell DNA, we judge that clone 19-30 contained about one copy of the *hprt* cDNA per cell and that clone 19-33 had multiple copies. Since the *Bam*HI fragments produced from clone 19-30 DNA appeared to be a bit shorter than the fragment from clone 19-33, we surmise that each was transformed by *hprt* cDNA clones that differed slightly in length; this suggests that there was more than one expressible *hprt* cDNA clone in the original cDNA library. Clone 19-22 lacked the *hprt* cDNA sequence and, as shown below, was probably a revertant of the mutation causing the HPRT$^-$ phenotype of A9 cells.

An examination of the HPRT protein produced by the clones that survived the HAT selection showed that two of the three—the same two that contained the human *hprt* cDNA segment—produced the human enzyme, and the clone that lacked the human *hprt* DNA produced an HPRT characteristic of the parent mouse L cells. Thus, cell extracts prepared from each of the cell clones were subjected to isoelectric focusing in polyacrylamide gel electrophoresis, and the position of active HPRT enzyme was located by in situ assay (3) (Fig. 7). Clearly, the A9 cell extracts had no detectable HPRT enzyme in the gel, whereas the three HAT-resistant clones had appreciable amounts of enzyme. Clones 19-30 and 19-33, however, produced HPRT with the unmistakable mobility of human HPRT, whereas clone 19-22 produced an enzyme characteristic of mouse L cells. Note that the amount of enzyme produced by clones 19-30 and 19-33 was lower than that in either the human or mouse cells or in clone 19-22, the HPRT revertant.

DISCUSSION

The goal of this work was to develop a vector system that would permit cloning of cDNAs based on the function they express in mammalian cells. This objective required a procedure for efficient cloning of full-length cDNAs (20) and a vector that would serve both for cloning cDNAs in bacteria and for expression of the cloned cDNA after transfer into mammalian cells (21). In the present experiments we have modified the procedure described earlier (21) so that the cDNA clone library can be incorporated into a λ phage

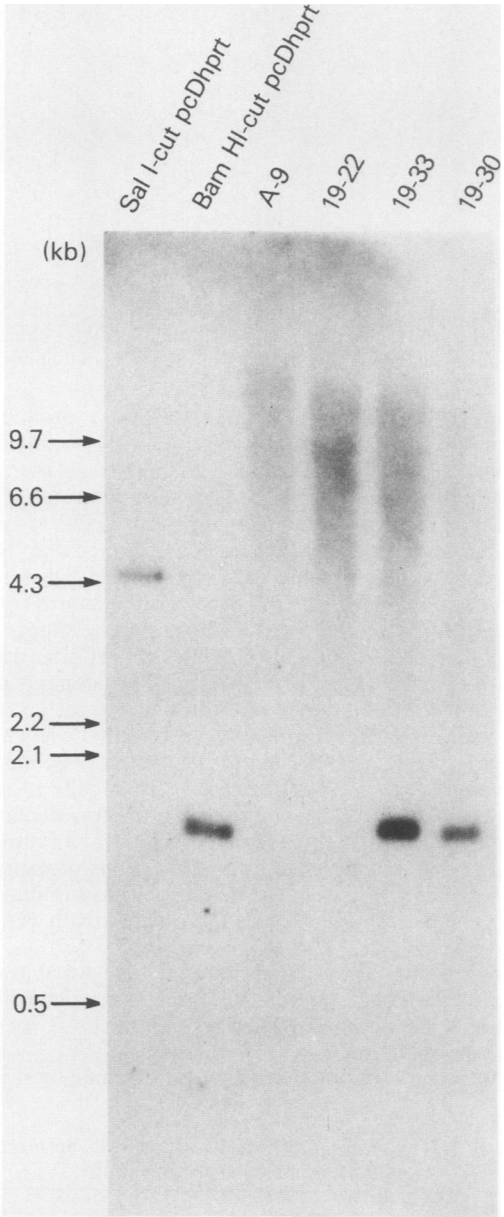

FIG. 6. Southern transfer analysis of DNAs obtained from cells that survived HAT selection. The total cell DNAs (15 μg each) prepared from A9 cells and the three HAT-resistant clones 19-22, 19-33, and 19-30 were digested to completion with *Bam*HI and electrophoresed in a 0.8% agarose gel with *Sal*I- or *Bam*HI-cleaved pcD*hprt* DNA (6 pg each) as markers. After transfer of the DNA to a nitrocellulose filter, the filter was hybridized with the ^{32}P-labeled, nick-translated *hprt* cDNA.

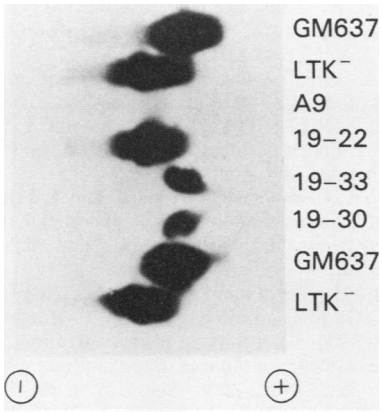

FIG. 7. Isoelectric focusing gel electrophoresis of extracts from HAT-resistant cells followed by assay of HPRT in situ. Cell extracts (10 μg of protein each) from GM637, LTK⁻, and A9 cells and the three HAT-resistant clones 19-22, 19-33, and 19-30 were electrophoresed in an isoelectric focusing polyacrylamide gel (pH 5.5 to 8.5) and assayed for HPRT activity by the method of Chasin and Urlaub (3).

genome and thereafter into mature λ bacteriophage. Since the λ vector genome contains a selectable marker that can be expressed in mammalian cells, transformants for the phage marker can be grown selectively and then screened for the function expressed from the cDNAs contained in the integrated phage DNA.

The key reasons lambda phage was chosen as the vector are the following. (i) Ishiura et al. (10) recently reported that lambda phage, after coprecipitation with calcium phosphate, transforms mammalian cells very efficiently for the herpes virus thymidine kinase marker. (ii) Lambda phage can accommodate large inserts of foreign DNA without impairing their ability to be propagated; this makes it possible to incorporate a genetic marker gene that can be selected for in mammalian cells and still leave enough space for insertion of the pcD recombinants. (iii) Lambda phage DNA can be packaged with high efficiency in vitro (6) so that it is relatively easy to transfer a cDNA library containing cDNAs in a wide range of sizes into the vector. (iv) Lambda DNA is large relative to the size of the cDNA transcription unit, so that integration of the vector DNA into the host DNA that accompanies stable transformations is very likely to leave the inserted pcD segment intact. That being the case, linkage of the replication origin and β-lactamase gene of the bacterial plasmid to the cDNA transcription units simplifies recovery of the pcD-cDNA recombinant by the transformed cells (see below).

Phage λ762 (18) was used in the construction of the λNMT vector because it grows well in most wild-type strains of *E. coli*, can accommodate relatively large inserts of foreign DNA, and has a useful array of restriction sites for the needed modifications (e.g., *Xho*I, *Hin*dIII, and *Sal*I). The essential modifications needed to convert λ762 to λNMT were (i) insertion of a *neo* gene that can be expressed in mammalian cells at the *Xho*I restriction site, and (ii) replacement of the 7-kb *Hin*dIII fragment containing the *supF* gene with a 0.5-kb *Hin*dIII fragment from SV40 DNA. The *Hin*dIII and *Sal*I restriction sites of λNMT are few enough to permit linearization of the pcD-cDNA library and insertion into the λNMT genome. Since λNMT contains only 38 kb of DNA, it grows poorly as a phage, a property which makes it ideal as a transducing vector; thus, recombinants that acquire an insert rapidly outgrow the vector phage. Inserts as large as 12 kb can be accommodated, and therefore pcD-cDNA plasmids containing up to 9 kb of cDNA can be propagated and cloned as phage.

The experiments in which the recombinant phage containing the pcD*hprt* plasmid were rare showed that the sequential selection protocol could detect a particular cDNA phenotype when 10^6 cells were transfected with a pool of cDNA containing 1 functional *hprt* clone in 10^5. This efficiency is comparable to that obtained by Ishiura et al. (10) with the Charon 4A phage containing the herpesvirus thymidine kinase gene. Further validation of the phage vector strategy and the sequential selection protocol was provided by successful isolation of two independent *hprt*$^+$ transductants by using an SV40-transformed fibroblast cDNA library which had been estimated previously (21) to contain 1 to 2 full-length *hprt* cDNA clones in 1.4×10^6 clones. Since the HPRT mRNA is among the least abundant class of mRNAs in the cells from which the cDNA library was prepared (two to three copies per cell [21]), this approach is applicable to the cloning of other rare mRNAs as cDNAs when a suitable selection is available for the cDNA function.

An important feature of the cloning and transduction protocol is the existence of a dominant selectable marker for mammalian cells in the transducing vector. The reconstitution experiments showed that direct selection for at least some phenotypes might not yield transductants even when the transducing cDNAs were present in the library. In the case of the *hprt* cDNA, we suspect that there is insufficient expression of the HPRT activity during the initial stages of the transformation event and before institution of the selection conditions. This is consistent with the observation (7) that the efficiency of transduction of cells to a particular phenotype is conditioned by the efficiency of the promoter transcribing the selectable function. Thus, it may be that efficient expression of the selectable function is required early so that sufficient quantities of the activity being selected for are present before institution of the lethal selection conditions. The sequential selection procedure provides a way for a potential transductant to express and accumulate the cDNA product before beginning the selection for this function. Once a steady state of product is reached it may be sufficient to permit growth in the selective medium. In the case of the two transductants obtained with the cDNA library there was only 20 to 30% of the HPRT activity present in wild-type or even revertant cells, but this was sufficient to confer resistance in the HAT selection medium. The initial selection for the dominant marker of the vector is also advantageous when no selection method exists for the cDNA-encoded function. In that case, the G418-resistant transductants provide a population of transduced cells that may then be screened for morphological or growth characteristics, the production of previously absent enzymes or metabolic products, or newly acquired surface markers by fluorescent-activated cell sorting.

There is an important distinction between the approach described here and those that use genomic DNA for the transduction. With our expression vector a cloned cDNA is expressed from a totally unrelated promoter, in the present case the SV40 early-region promoter, but in principle from any promoter. As a result, expression of the cDNA differs qualitatively and quantitatively from that of the corresponding cellular gene; it could be constitutive or inducible and at either reduced or markedly elevated levels. Furthermore, judicious choice of promoters and enhancers or of processing signals incorporated into the vector DNA could influence the level of expression in different cells. The possibility of elevated expression in heterologous cells provides an opportunity to search for genes whose enhanced or constitutive

expression in such heterologous hosts confers unexpected phenotypes, for example, the expression of cryptic or potential oncogenes. Thus, it may be possible to search for genes expressed in a highly regulated way in one cell type by employing regulatory signals that are appropriate and functional in other cell types.

It is important to emphasize that our present procedure identifies cells that have been transduced to a particular phenotype by cDNAs that have become integrated into the cellular genome. For the chemical characterization of the transducing cDNA and ultimate isolation of the genomic equivalent, it is of course necessary to recover the transduced cDNA sequence. This could be done by constructing a lambda phage or cosmid library from the total cellular DNA of the stable transformants and using the SV40 sequences that flank the cDNA insert as probes for plaque or colony hybridizations to identify the relevant phage or cosmids. Such an approach would also require a second round of transductions with the recovered DNAs to identify the cDNA segments being sought. Such screening procedures would be tedious and time-consuming. An alternative is provided by the presence of the pBR322 replication origin, the β-lactamase gene, and the SV40 replication origin in the integrated λNMT-pcD recombinant. Cell fusion between the transduced cells and COS cells (9) has been used by Breitman et al. (2) to recover the pSV2 molecule from transformed cells. Large T antigen and other permissive factors provided by COS cells drive the initiation of replication at the integrated SV40 origins and promote excision of the replicated DNA as extrachromosomal circular DNA. Since such plasmids are likely to also contain the flanking pBR322 *ori* and β-lactamase gene, they can be cloned by transformation in *E. coli*. In the present instance, we obtained several *E. coli* transformants with the low-molecular-weight DNA recovered after fusing the $HPRT^+$ transformed cells with COS cells; the plasmids obtained from such *E. coli* cells contained the expected *hprt* cDNA insert and flanking sequence.

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