High-Efficiency Transformation of Mammalian Cells by Plasmid DNA

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We describe a simple calcium phosphate transfection protocol and *neo* marker vectors that achieve highly efficient transformation of mammalian cells. In this protocol, the calcium phosphate-DNA complex is formed gradually in the medium during incubation with cells and precipitates on the cells. The crucial factors for obtaining efficient transformation are the pH (6.95) of the buffer used for the calcium phosphate precipitation, the CO_2 level (3%) during the incubation of the DNA with the cells, and the amount (20 to 30 μg) and the form (circular) of DNA. In sharp contrast to the results with circular DNA, linear DNA is almost inactive. Under these conditions, 50% of mouse L(A9) cells can be stably transformed with pcDneo, a simian virus 40-based *neo* (neomycin resistance) marker vector. The NIH3T3, C127, CV1, BHK, CHO, and HeLa cell lines were transformed at efficiencies of 10 to 50% with this vector and the *neo* marker-incorporated pcD vectors that were used for the construction and transduction of cDNA expression libraries as well as for the expression of cloned cDNA in mammalian cells.

The recent development of techniques for DNA transfection of cultured cells has provided powerful methods for examining the function of various parts of complex mammalian genes. In fact, novel transcriptional regulatory elements, as well as various RNA processing and translational signals, have been discovered by using DNA transfection of tissue culture cells (1, 2, 6, 7, 14, 28, 30). It has also been possible to isolate genes of interest after transfecting cells with a pool of genomic DNA and selecting for appropriate transformants by using genetic complementation or altered phenotype. Various mammalian genes including thymidine kinase (27), hypoxanthine guanine phosphoribosyltransferase (HPRT) (11), thymidylate synthase (36), transferrin receptor (15), DNA repair gene (37), and new oncogenes (29, 32, 33) have been isolated by following this approach.

Nearly a dozen transfection techniques have been devised, all of which involve the use of either calcium phosphate or DEAE-dextran (or its analogs) as a carrier to deliver DNA into cells (5, 9, 16, 19, 20, 26). In some methods, osmotic shock or treatment with lysosomal inhibitors is used to enhance the transfection efficiencies (19, 26). Recently a method involving the use of high-voltage electric pulses to create pores in membranes has been devised for delivering DNA into cells (22).

These transfection methods are quite useful for examining transient expression of DNA, but they are inefficient for stable transformation. With few exceptions (8), the transformation frequencies obtained by these methods are relatively low, ranging from 0.001 to 1% (5, 8, 9, 16, 19, 22, 26), depending on the cell line used as recipient. These low transformation frequencies have prevented the use of a variety of interesting cell mutants as hosts for genetic complementation or for cloning of cDNA on the basis of its expression.

We have recently found extremely efficient conditions for calcium phosphate-mediated transfection of cells. Under these conditions, many common cultured mammalian cell lines are stably transformed with efficiencies of 10 to 50% by pcD-derived cDNA and *neo* dual expression vectors.

MATERIALS AND METHODS

Chemicals. *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) was obtained from Calbiochem-Behring; $CaCl_2$ was purchased from Mallinckrodt, Inc., or J. T. Baker Chemical Co.

Transfection buffers. A stock solution of 2.5 M $CaCl_2$ was prepared, filter sterilized through a 0.45-μm-pore-size nitrocellulose filter (Nalge), and stored at −20°C. Then 2× BES-buffered saline (2× BBS) containing 50 mM BES (pH 6.95), 280 mM NaCl, and 1.5 mM Na_2HPO_4 was prepared, filter sterilized, and stored at −20°C. The pH was adjusted with HCl at room temperature.

Cell culture. A9 (17), a hypoxanthine guanine phosphoribosyltransferase (HPRT)-deficient mouse L cell, was used as the recipient to test the variables in the transfection assays. Other established cell lines used were CV1 (American Type Culture Collection, Rockville, Md.), an African green monkey kidney cell; CHO-K1 (American Type Culture Collection); SN10 (3), a subclone of BHK-21 (cl 13); HeLa (American Type Culture Collection); C127 (18), a mouse mammary tumor cell; NIH 3T3 (31); MB66MCA (cl 13) (31), a methylcholanthrene-transformed C3H10T1/2 line; GM637 (National Institute of General Medical Sciences Human Genetic Mutant Cell Repository), a simian virus 40 (SV40)-transformed human fibroblast; and P3X63-AG8.653 (American Type Culture Collection), a mouse myeloma cell line. All cells were maintained in Dulbecco modified Eagle medium (DMEM) (α-MEM for CHO and RPMI 1640 for mouse myeloma) supplemented with 10% fetal calf serum, penicillin, and streptomycin in a 5% CO_2, 35°C Forma incubator.

Plasmid DNA. Plasmid DNA was prepared by the lysozyme-Triton procedure (13) followed by two cycles of CsCl equilibrium density gradient centrifugation and repeated phenol extractions after addition of 1% sodium dodecyl sulfate (*Escherichia coli* proteins are toxic to cells).

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Transfection of cells. Cells were transfected by the following protocol. Exponentially growing cells were trypsinized, seeded at 5×10^5 cells per 10-cm plate, and incubated overnight in 10 ml of growth medium. Then 20 to 30 μg of plasmid DNA was mixed with 0.5 ml of 0.25 M $CaCl_2$, 0.5 ml of 2× BBS was added, and the mixture was incubated for 10 to 20 min at room temperature. Calcium phosphate-DNA solution (1 ml) was added dropwise to the plate of cells, and the mixture was swirled gently and incubated for 15 to 24 h at 35°C under 2 to 4% CO_2. The medium was removed, and the cells were rinsed twice with growth medium, refed, and incubated for 24 h at 35 to 37°C under 5% CO_2. The cells were split at an appropriate ratio (>1:10) and incubated for an additional 24 h before selection for stable transformants was begun.

Transformation efficiency. To determine the frequency of stable transformation, cells were transfected with various amounts (10 to 40 μg) of pcDneo, pcD2hprt, or pcD2tkhprt as described above. The transfected cells were split and replated at 10^3 cells per 10-cm plate 24 h prior to selection. For each transfection, a total of four plates of 10^3 cells were used, two for selection and two for a plating efficiency assay. To determine the number of Neo$^+$ transformants, cells were selected for 2 to 3 weeks in growth medium containing 400 μg of G418 medium (100% potency) per ml. To determine the plating efficiency, cells were maintained in growth medium for 1 week; the colonies were then stained and counted. The transformation efficiency, expressed as percent transformation, was calculated by dividing the average number of Neo^r colonies (greater than 100 cells) by the average number of colonies that grew in nonselective growth medium and multiplying the result by 100. The plating efficiencies of the cells tested were 10 to 70%.

The transformation frequency of the mouse myeloma cell was determined as follows. After they were transfected as described above, cells were replated in 24-well dishes at 100 cells per well and selected in G418 medium (400 μg/ml) for 3 weeks. Then the number of wells containing growing cells was counted. The transformation frequency was calculated by dividing the number of positive wells by the total number of cells selected.

Construction of pcDneo. The *neo* coding region was excised from pSV2neo (35) by *Bam*HI and *Bgl*II digestions and inserted into the *Bam*HI sites of the pcD vector (24).

Construction of pL2 plasmids. The 3' noncoding region (about 300 base pairs) of the *neo* gene was removed from pcDneo by *Asu*II-*Bam*HI double digestion followed by blunt ending and cyclization. The *neo* transcriptional unit was excised from the truncated pcDneo by *Hin*dIII-*Dra*I digestion (which creates blunt ends). pL1 (24) was linearized with *Hin*dIII. Half of the *Hin*dIII-cut pL1 DNA was then digested with *Eco*RI, and the pBR segment released was purified by agarose gel electrophoresis. The other half was treated with *E. coli* DNA polymerase I to blunt end, and the *Hin*dIII termini was digested with *Eco*RI. The fragment containing the SV40 promoter and the splice junction was purified. pL2 was constructed by a three-piece ligation of the *neo* fragment (*Hin*dIII-blunt ends), the pBR segment (*Hin*dIII-*Eco*RI ends), and the fragment containing the SV40 promoter (*Eco*RI-blunt ends).

pL2 was converted to pL2tk by replacing the segment containing the SV40 promoter and the 19S splicing junctions with the herpes simplex virus thymidine kinase promoter *Hin*dIII-*Bgl*II fragment (21). pL2 was digested with *Hin*dIII and *Pvu*II (a unique *Pvu*II site resides in the *neo* gene), and the large fragment was purified by agarose gel electrophore-

sis. The 5' end of the *neo* gene was excised from pSV2neo by *Bgl*II-*Pvu*II double digestions. The *tk* promoter (*Hin*dIII-*Bgl*II ends), the pL2 fragment (*Hin*dIII-*Pvu*II ends), and the 5' region of the *neo* gene (*Bgl*II-*Pvu*II) were ligated together to yield pL2tk.

RESULTS

High-efficiency transfection. The transfection protocol we used is a modification of the standard calcium phosphate methods for DNA (5, 8, 9, 16) and bacteriophage particle (10) transfection (see Materials and Methods). It is simple, yet extremely efficient. Plasmid DNA was mixed with calcium chloride and 2× BBS at pH 6.95, a pH value considerably lower than that used in the standard methods (5, 8, 9, 16). The mixture was added directly to medium in a dish containing cells and incubated overnight under a low (2 to 4%) CO_2 atmosphere. Because of the low pH, the calcium phosphate-DNA complex forms gradually in the medium and precipitates on cells during the overnight incubation. Transfected cells were incubated in growth medium, split at an appropriate ratio, and selected by the standard protocol (35).

The test plasmid used was pcDneo. pcDneo was constructed by inserting the *Bgl*II-*Bam*HI fragment of the *neo* gene in the *Bam*HI site of the pcD vector (24). The presence of the bacterial promoter in the *neo* fragment decreases the translation of *neo* in mammalian cells, perhaps owing to the presence of several ATG codons in the promoter region (35). It was therefore removed during construction of the pcDneo plasmid. The *neo* gene is thus expressed more efficiently in pcDneo than in the pSV2neo plasmid, which retains the bacterial promoter (35).

A9 cells were used to optimize each parameter influencing transformation efficiency. The amount and the form of DNA, the pH of the 2× BBS, and the level of CO_2 in the incubator during transfection were found to be crucial factors for efficient stable transformation.

Figure 1 illustrates a typical DNA dose-response curve obtained with A9 cells. The curve is very steep: at the optimum DNA concentration (about 20 μg of DNA per 10 ml of medium in a 10-cm dish) the transformation frequency was more than 50%. With either 10 or 30 μg of DNA, the transformation frequency decreased about 5- to 10-fold. The optimum amount of DNA varied from 10 to 30 μg depending on the recipient cell line and the preparation of DNA. We observed that with increasing amounts of DNA, there was a dramatic change in the quality of the calcium phosphate-DNA precipitates that developed during the overnight incubation with cells (Fig. 2). The precipitates formed with 10 μg of DNA were very coarse and easily visible. With 30 μg of DNA or more, the precipitates were extremely fine and almost invisible under a microscope at low magnification (×40). Use of 20 μg of DNA resulted in fine, visible precipitates.

Linear DNAs such as *Hin*dIII-cut pcDneo or λNMT (25), a λ phage DNA containing a *neo* marker gene identical to that in pcDneo, were almost inactive at all amounts tested; transformation frequencies were less than 1%. However, there were no apparent differences in the appearance of the precipitates formed with circular and linear DNAs.

As mentioned above, pSV2neo was about fivefold less active than pcDneo, and in general, Neo^r colonies obtained with pSV2neo were smaller than those obtained with pcDneo. Nevertheless, the transformation efficiency (10%) obtained with pSV2neo in this study was at least 50-fold higher than those obtained by the standard method (8, 35).
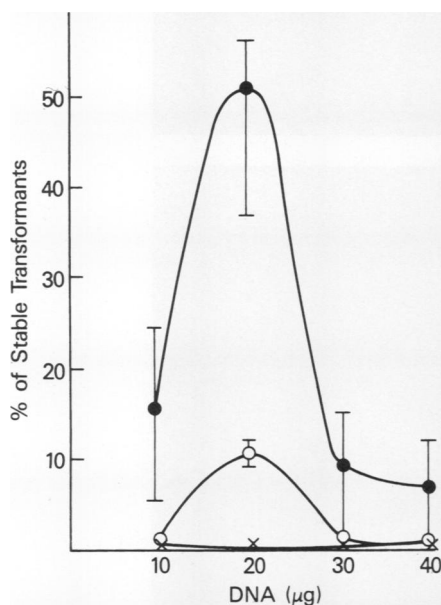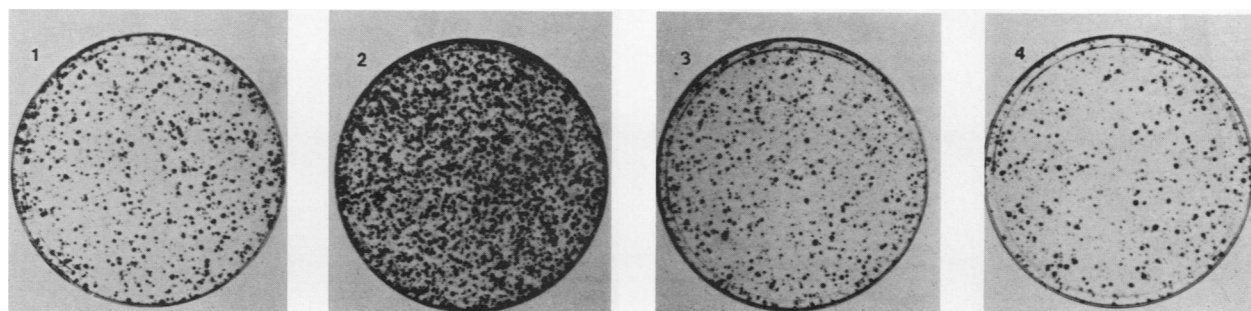
FIG. 1. Effect of the amount and form of DNA on transformation frequency. Top: A9 cells were transfected with various amounts of pcDneo (10 μg [panel 1], 20 μg [panel 2], 30 μg [panel 3], and 40 μg [panel 4]), trypsinized, and replated at 10^4 cells per plate. The cells were selected in G418 medium for 2 weeks and stained with Giemsa. Bottom: transformation by pcDneo (average of four separate experiments) (●); transformation by pSV2neo (average of two separate experiments) (○); transformation by HindIII-cut pcDneo or λNMT (×). Vertical bars indicate the highest and lowest values obtained. The transfection conditions and frequency assay are as described in Materials and Methods.

For efficient transformation, the pH of the 2× BBS buffer must be set accurately. When 2× BBS buffers of various pHs were tested, the optimum pH for transformation was found to be around 6.95 (Fig. 3A). At pHs of 7.05 to 7.15, which are used in the standard method (5, 8, 9, 16), the transformation frequency was severalfold lower.

The CO_2 level in the incubator during the transfection is also important. Although 5 to 10% CO_2 is commonly used for cell growth, the optimum CO_2 level for stable transformation was 3% (Fig. 3B). Under these conditions, the medium was slightly basic (pH 7.6 to 7.7) after overnight incubation.

Doubling the amount of medium in the culture plate allowed twice as much DNA to be applied to cells without changing the property of the precipitate. However, this had no significant effect on transfection efficiency under optimal conditions.

The transformation efficiency was relatively insensitive to the temperature of the incubator during the transfection. At 34, 35, 36, and 37°C there was no significant difference in the transformation efficiency. Within the range of the various pHs and the CO_2 levels examined, the optimum DNA concentration remained constant.

The transformation frequencies of various common mammalian fibroblast or epithelial cell lines were determined under the most favorable conditions described above (Table 1). The amount of pcDneo was optimized for each cell line. As with the A9, C127, CV1, NIH 3T3, BHK, CHO and

HeLa lines, cells were transformed at frequencies of 10 to 50%; even GM637, an SV40-transformed human fibroblast cell line, which is a relatively poor host cell, was transformed at a frequency of 3% or more (see the section on transfection with pcD2hprt or pcD2tkhprt). We also tested one mouse myeloma cell line as a transfection host. The transformation frequency of this cell line was far below those of the anchorage-dependent cell lines examined. Cotransfection of A9 cells with 2 μg of pcDneo and 18 μg of pcDhprt (12) resulted in a ca. fivefold decrease in transformation frequency (10%). In this experiment, the optimum total DNA amount was unchanged (20 μg), and a similar dose-response curve was obtained.

The neo transformants generated under these conditions grew continuously in the presence of G418 medium. Analysis of the Hirt supernatant (4) and the genomic DNA of the transformed A9 cells by Southern blotting (34) showed that all of the pcDneo sequences were integrated into high-molecular-weight DNA, and no free pcDneo molecules were detected in the Hirt supernatant. Similar analysis of the CHO transformants following restriction enzyme digestion indicated that three to five copies of pcDneo were integrated in the transformants.

pcD2 vectors. To take full advantage of this protocol, we have modified the pcD vector (24) by inserting into it the neo transcriptional unit driven by the SV40 or herpes simplex virus thymidine kinase promoter. The resulting pcD2 vector contains the neo transcriptional unit joined in tandem to the cDNA transcriptional unit (Fig. 4). The pcD2tk vector has the same structure, but the herpes simplex virus thymidine kinase promoter (21) is used to express the neo gene. These vectors are designed to be used for the construction and transduction of cDNA expression libraries (23, 24), as well as expression of cloned cDNA in mammalian cells. pL1 or pL1tk and pcDV1 provide DNA reagents for constructing cDNA libraries (24) or inserting cloned cDNA in the pcD2 vector.

To test the function of these vectors and verify the high efficiency of the transfection protocol with different plasmids, the human hprt cDNA cloned in the pcD vector (12) was used to prepare pcD2hprt and pcD2tkhprt (Fig. 4, X is
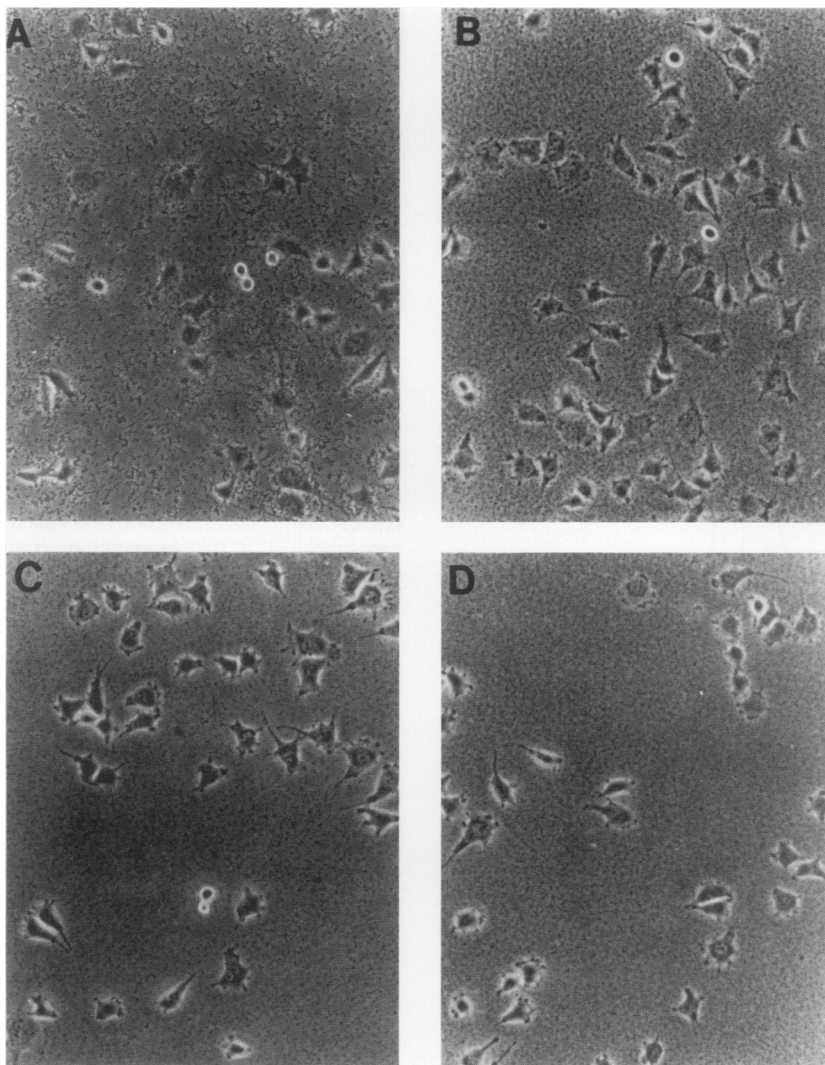
FIG. 2. Effect of the DNA amount on the properties of calcium phosphate-DNA precipitates. After transfection of A9 cells with 10 μg (A), 20 μg (B), 30 μg (C), or 40 μg (D) of pcDneo, photographs were taken under a phase-contrast microscope at ×200 magnification.
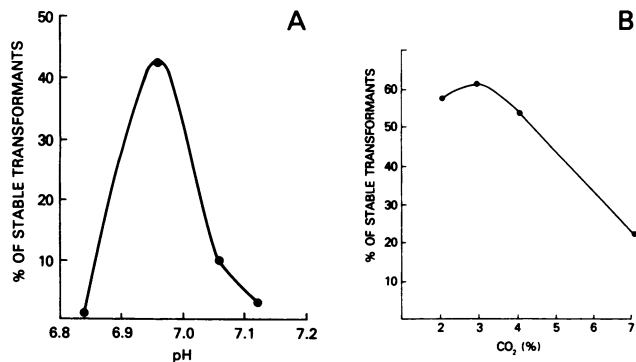

FIG. 3. Effects on transformation frequency of the pH of the 2× BBS and the CO_2 level in the incubator. The transfection conditions are as described in Materials and Methods, except that the pH of the 2× BBS (A) or the CO_2 levels (B) in the incubator varied. The optimal amount of DNA was 20 μg for all pHs tested.

hprt) and these plasmids were used for transfection of various cells. Most of the cell lines tested became Neo^r of frequencies similar to those obtained with pcDneo (Table 1). A9 cells were transformed twofold less efficiently, while GM637 cells were transformed severalfold more efficiently with these vectors. The transformation frequencies obtained with pcD2 and pcD2tk were similar for most of the cell lines tested; the pcD2 vector seems to transform HeLa cells a little more efficiently than the pcD2tk vector.

The levels of expression of cDNA obtained with the pcD2 and the pcD vector were comparable. Transfection of A9 cells with pcDhprt (12) and pcD2hprt followed by HAT selection (17) yielded the same number of HPRT-positive colonies (Fig. 5C and D). The number of HPRT-positive colonies, however, was always about 10-fold lower than the number of *neo*-positive colonies obtained in the same experiments, regardless of whether pcDneo and pcDhprt were compared with each other or pcD2hprt was compared with itself (compare Fig. 5A with C and B with D). This suggests that a relatively high level of expression of HPRT is required for the cells to survive HAT selection. The transformation

TABLE 1. Transformation efficiencies of various cell lines

Cell line	% of stable *neo* transformants		
	pcDneo	pcD2hprt	pcD2tkhprt
A9	50 ± 16[a] 10[d]	21 ± 12[b]	17 ± 3[c]
C127	32	−[e]	−
NIH 3T3	16	14, 15	12, −
CV1	20	43[f], 40[f]	53[f], −
SN10 (BHK)	47	31, 17	25, 40
CHO	15	20, 11	12, −
HeLa	17	24, 42	11, 9
MB66MCA (methylcholanthrene-transformed C3H10T1/2)	6	11, −	
GM637 (SV40-transformed human fibroblast)	3[g]	16[g], 29[g]	20[g], 15[g]
P3X63-AG-8.653 (mouse myeloma)	0.03	0.03	

[a] Average value and standard deviation of the results of seven separate experiments.
[b] Average value and standard deviation of the results of five separate experiments.
[c] Average value and standard deviation of the results of three separate experiments.
[d] Cotransfection of 2 μg of pcDneo and 18 μg of pcDhprt (12) followed by *neo* selection.
[e] −, Not determined.
[f] G418 selected at 37°C (selection at 35°C resulted in an approximately twofold decrease in transformation frequency). The results with pcD2hprt, and pcD2tkhprt are from two separate experiments.
[g] A total of 10⁴ cells were selected. The optimal amount of pcDneo was 20 μg for A9, CHO, GM637, HeLa, and P3X63 cells and 30 μg for BHK, NIH 3T3, CV1, C127, and MB66 cells; that of pcD2hprt was 20 μg for HeLa, GM637, CV1, and P3X63 cells, 30 μg for A9, NIH 3T3, CHO, and BHK cells, and 40 μg for MB66 cells; and that of pcDtkhprt was 20 μg for HeLa and CV1 cells and 30 μg for A9, BHK, NIH 3T3, CHO, and GM637 cells.

frequency to HAT resistance was 1.5 to 2%, a value about 20-fold higher than that previously obtained by using the standard protocol (12).

Southern blotting analysis (34) of genomic DNAs following restriction enzyme digestion, and secondary genomic transfection followed by recovery of integrated plasmids by COS cell fusion (4), indicated that four to six copies of the pcD2 molecules were integrated in the CHO and C3H transformants and that approximately 30% of the integrated molecules were at least dimers.

DISCUSSION

To achieve efficient stable transformation of mammalian cells by DNA transfection, two factors must be considered: efficient delivery of DNA into the cell nuclei to promote its integration into the host chromosome and sufficient amounts of expression of the transduced gene to allow the cell to survive and grow in the course of selection. Taking these factors into consideration, we have developed a simple procedure involving the use of calcium phosphate-mediated DNA transfection and *neo* marker vectors, which achieve extremely efficient transformation of mammalian cells. Transformation efficiencies of 10 to 50% were obtained with most fibroblast and epithelial cell lines tested. The unique feature of our procedure is the low pH of the 2× BBS buffer. With this buffer, calcium phosphate-DNA precipitates develop gradually in the medium during overnight incubation with the cells rather than rapidly in the solution after mixing. This slow development of the calcium phosphate-DNA complex in the medium seems to be the major factor contributing to the extremely high transformation efficiency.

We do not know the reason for the enhancement; perhaps the structure of the calcium phosphate-DNA complex promotes uptake by a larger number of cells, promotes more efficient uptake by cells, or promotes preservation of the DNA while it is en route to the nucleus. Since the copy number of the integrated plasmid did not seem to be increased, the first reason (uptake by a larger number of cells) is the most likely.

In addition to the low pH, the amount of DNA and the level of CO_2 (and possibly a component of the medium) are important for the formation of the appropriate calcium phosphate-DNA complex. There is a dramatic change in the nature of the calcium phosphate precipitate with increasing amounts of DNA. A transition from coarse to fine precipitates occurred at the optimum DNA concentration (Fig. 3).

In the present study, we used pcDneo, the pcD vector with the insertion of the *neo* coding sequence, and its derivatives that were developed for use in the construction and transduction of cDNA expression libraries or for the expression of cloned cDNA in mammalian cells. These vectors yielded 5 to 10 times more transformants than did pSV2neo and pcDhprt, the original *neo* marker vector and a *hprt* selectable marker vector. The pcDneo vector plasmid and its derivatives stably transformed mouse, CHO, BHK, and human cell lines at efficiencies of 10 to 50%, levels at least 50- to 100-fold higher than previously achieved (5, 8, 9, 16, 19, 22, 26) and comparable to those obtained by infection with retroviral vectors. However, linear DNAs such as *Hind*III-cut pcDneo and λNMT (25), which contain an identical *neo* transcription unit, transform only 1% of cells at most. The reason for the poor transformation by linear DNA is not clear. Linear DNA may be susceptible to nucleases in the medium during the formation of the calcium phosphate complex or during transport of the DNA to the nucleus following endocytosis. Alternatively, linear DNA may be poorly converted to the calcium phosphate complex taken up by the cells.

The transfection conditions described in this paper have been optimized for mouse A9 cells, DMEM with 10% fetal bovine serum, and pcDneo. Although the transfection procedure outlined here works well for a variety of cells, some other media (e.g., α-MEM), and the pcD2 vector, the optimal conditions may vary with different cells, medium, amount of DNA, and CO_2 level. We did observe slight shifts in the optimum amount of DNA (often 15 or 25 μg in other experiments) among different preparations; therefore the method may require fine tuning for each cell line, medium, and DNA. In addition to fibroblastic and epithelial cell lines, hematopoietic cells can be stably transformed by this method, but far less well than anchorage-dependent cells (Table 1).

Enhanced transformation frequency is neither unique to the *neo* gene nor specific to transfection by homogenous DNA. High-efficiency transformation (2 to 10%) was obtained with pcDhprt by using HAT selection or by cotransfection with 2 μg of pcDneo and 18 μg of carrier pcD DNA followed by *neo* selection.

It is useful to know whether the method is efficient for transient expression. Preliminary observations suggest that the level of expression obtained by this method is almost comparable to that obtained by the DEAE-dextran method combined with chloroquine treatment (19).

One of the goals of our present studies is to develop a system for direct cloning of cDNAs that is based on the function of the proteins they encode. We have already developed a method and vector system for the construction
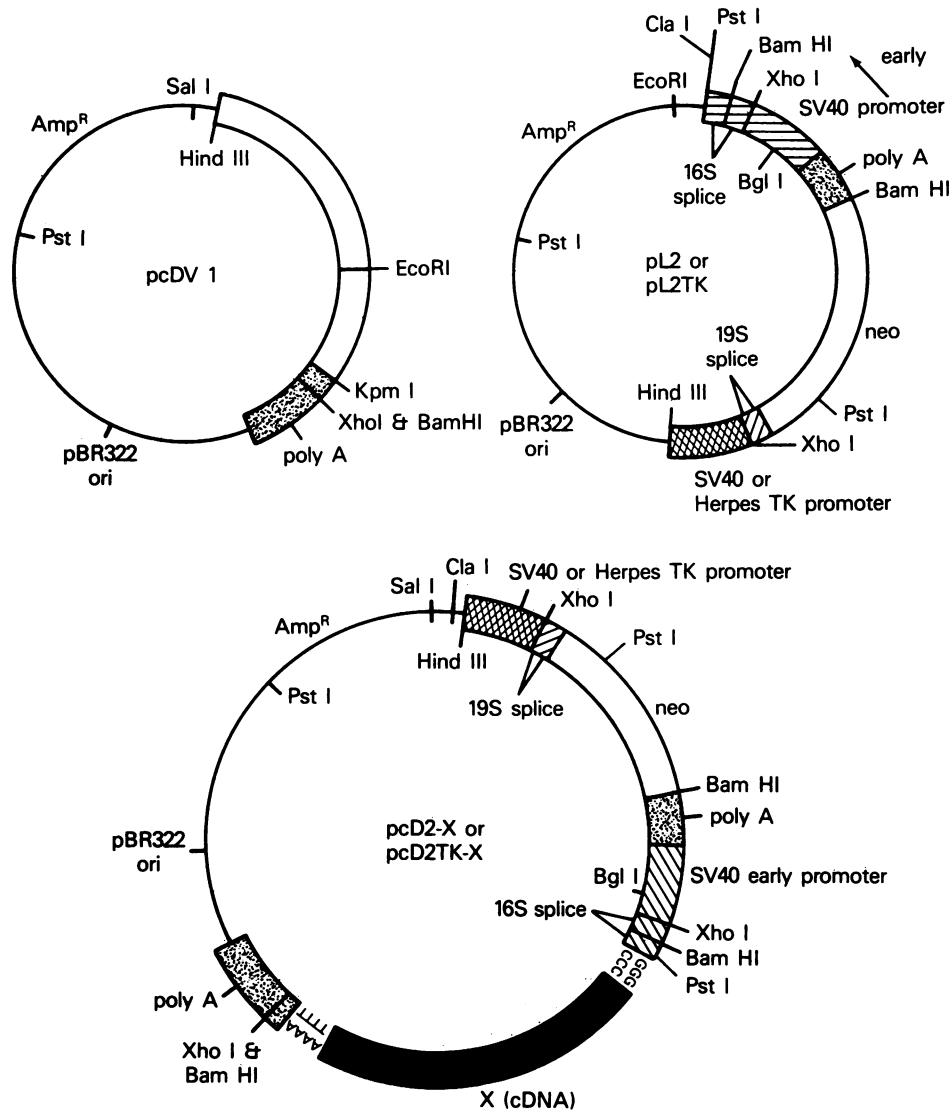
FIG. 4. Structure of the pcD2 and pcD2tk vectors and their precursor plasmids, pcDV1, and pL2 and pL2tk. The principal elements of the pcD2 vectors are (i) a segment of the *neo* transcriptional unit (see Materials and Methods) with the SV40 early promoter (pcD2) or the herpes simplex virus thymidine kinase promoter (pcD2tk) and (ii) the entire pcD vector segment (24), which contains an SV40-based transcriptional unit with cDNA (▬) inserted through dG-dC and dA-dT bridges in the cloning operation (23, 24) and the pBR322 segment with the β-lactamase gene (Amp^r) and the plasmid replication origin (pBR322 ori) (———). In the pcD2tk recombinant, the 19S splice junction upstream of the *neo* gene is eliminated. pcDV1, pL2, and pL2tk are precursor plasmids for the preparation of the vector primer and the linker DNA (23, 24). pcDV1 is shared with the original pcD system. Since a *Pst* site is present in the *neo* coding region, the linker DNA was prepared by *Cla*I digestion followed by blunt-ending with *E. coli* DNA polymerase I and oligo(dG)-tailing with terminal transferase.

of full-length cDNA expression libraries for mammalian cells (23, 24). We subsequently developed a phage vector for transducing cDNA libraries into mammalian cells (25). This transducing system, which relies on calcium phosphate-mediated phage particle transfection, however, has been limited in its application to a variety of cells because of its low transduction efficiency. The typical transformation frequency of A9 cells obtained with the phage system is 0.5 to 1%, compared with 15 to 50% obtained with pcDneo plasmid DNA in the present study. To take full advantage of the procedure for cDNA expression cloning, we have constructed pcD2 vectors with the *neo* marker gene that permits direct cloning and expression of cDNA. These vectors transform cells to G418 resistance as efficiently as pcDneo does and express cDNA inserts as efficiently as the original pcD vector does. We are currently constructing cDNA libraries with the pcD2 vector and exploring the feasibility of direct cloning of cDNA by transducing the libraries into cells and then screening or selecting cell colonies on the basis of the changes in their phenotypes.
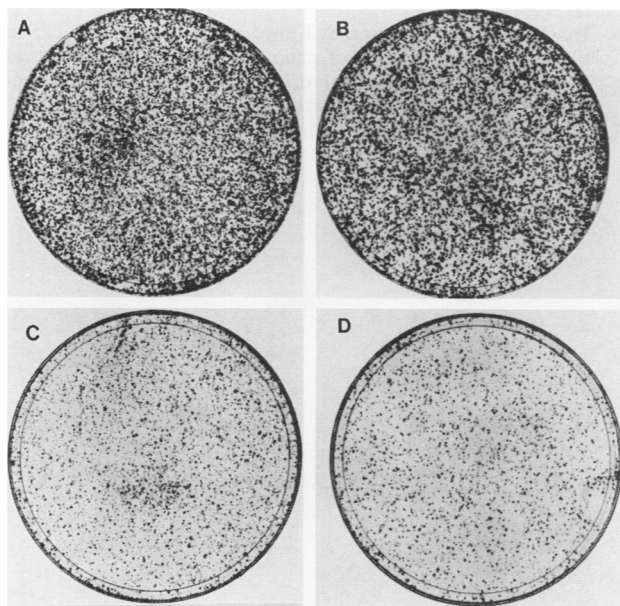
ACKNOWLEDGMENTS

FIG. 5. Comparison of pcD2hprt with pcDneo and pcDhprt in transformation efficiencies. A9 cells were transfected with the optimum amount (20 μg) of pcDneo, pcD2hprt, or pcDhprt (12), trypsinized, and replated at 20,000 cells per plate. The cells were selected in G418 or HAT medium (17) for 2 weeks and stained with Giemsa. (A) Transfected with pcDneo and selected in G418 medium; (B and D) transfected with pcD2hprt, split, and selected in G418 medium (panel B) or HAT medium (panel D); (C) transfected with pcDhprt and selected in HAT medium.

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