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Efficient screening of retroviral cDNA expression libraries

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ABSTRACT **Expression cloning of cDNAs was first described a decade ago and was based on transient expression of cDNA libraries in COS cells. In contrast to transient transfection of plasmids, retroviral gene transfer delivers genes stably into a wide range of target cells. We utilize a simple packaging system for production of high-titer retrovirus stock from cDNA libraries to establish a cDNA expression cloning system. In two model experiments, murine interleukin (IL)-3-dependent Ba/F3 cells were infected with libraries of retrovirally expressed cDNA derived from human T-cell mRNA or human IL-3-dependent TF-1 cell line mRNA. These infected Ba/F3 cells were selected for the expression of CD2 by flow cytometry or for the α subunit of the human IL-3 receptor (hIL-3Rα) by factor-dependent growth. CD2 (frequency, 1 in 10^4) and hIL-3Rα (frequency, 1 in 1.5×10^5) cDNAs were readily detected in small-scale experiments, indicating this retroviral expression cloning system is efficient enough to clone low-abundance cDNAs by their expression or function.**

Expression cloning is a powerful tool with which to isolate a cDNA of interest when a phenotypic function of a protein is known but its amino acid sequence is not known. For instance, many cytokine cDNAs were cloned by assaying the growth-promoting activity of culture supernatant of COS cells transiently transfected with subdivided cDNA libraries (1). The principle of the first cDNA expression cloning system (2) was to amplify expression vectors carrying the simian virus 40 (SV40) replication origin (ori) in mammalian cells stably expressing tumor antigen (T antigen) [i.e., a transformed African green monkey kidney cell line, COS (3)]. The presence of the SV40 large T antigen in COS cells allows replication of SV40 ori-containing plasmids, thus amplifying expression of the cDNA on the plasmid (2, 3). A notable alteration to this strategy was introduced by Seed and Aruffo (4), in which transfected COS cells were selected for the expression of a known antigen by panning transfected cells on antibody-coated plates. Genes encoding many surface markers for which antibodies were available have been cloned in this way (4, 5). Alternatively, the cell sorter was also utilized to collect COS cells that express a cDNA of interest (6). The cDNAs for cytokine receptors have been also isolated from cDNA libraries by screening for cytokine binding (7–9). In addition, a transcription factor GATA-1 cDNA was isolated by COS-cell expression cloning using gel mobility shift assay as a screening procedure (10). Thus, expression cloning of genes expressing a desired protein or activity is limited only by the ingenuity of the experimental design.

Despite many applications, most conventional expression cloning systems still suffer from the need for transient amplification of plasmids in particular cell lines expressing the SV40 (or polyoma) large T antigen. First of all, the function of the target gene has to be suited to transient detection. Moreover, target cells are restricted to cells that allow SV40 large T antigen-based amplification and to cells in which the transfection efficiency is high (>10%). Approaches using transient expression system in COS cells have obvious limitations in searches for proteins with various functions in specialized cell types.

To overcome these limitations, we designed a high-efficiency retrovirus cDNA library system for expression cloning (Fig. 1). We constructed cDNA libraries in a retroviral vector pBabeX, a derivative of a Moloney murine leukemia virus-based vector, pBabe-puro (11) (Fig. 2). Retroviruses representative of the cDNA library were produced by using a transient retrovirus packaging system (12), with modifications. The supernatants of the packaging cell line, containing high-titer retroviruses ($>3 \times 10^6$ plaque-forming units/ml), were then used to infect hemopoietic cells, and infected cells were selected for expression of cDNA of interest. Here we demonstrate that relatively rare cDNAs encoding the CD2 antigen and the human interleukin 3 receptor α (hIL-3Rα) can be easily and effectively isolated (Table 1).

MATERIALS AND METHODS

Cytokines and Cell Lines. Purified recombinant human granulocyte/macrophage colony-stimulating factor (hGM-CSF) produced in *Escherichia coli* was provided by R. Kastelein (DNAX). Recombinant murine (m) IL-3 was derived from silkworm recombinant expression systems. *E. coli*-derived recombinant hIL-3 was generously provided by Monica Tsang (R&D Systems). A retrovirus packaging cell line BOSC23 for ecotropic retroviruses (ATCC CRL 11554) (12) was maintained in Dulbecco's modified Eagle's medium containing 10% (vol/vol) fetal calf serum (DMEM/10% FCS) and GPT selection reagents (Specialty Media, Lavellette, NJ). The cells were transferred into DMEM/10% FCS without GPT selection reagents 2 days before transfection. NIH 3T3 cells were maintained in DMEM/10% FCS. A murine pre-B-cell line, Ba/F3, and a human erythroleukemic cell line, TF-1, were cultured as described (13, 14).

Retroviral Vector and Constructs. pBabe-neo and pBabe-puro vectors (11) were kindly provided by J. P. Morgenstern (Ariad Pharmaceutical, Cambridge, MA). We modified the pBabe-puro vector by disrupting the *Not* I site, deleting the SV40 promoter and the puromycin-resistance gene (*Cla* I–*Sal* I fragment), and changing the multicloning sites to include *Eco*RI and *Not* I sites for construction of unidirectional libraries (Fig. 2). The new sequence of the polylinker is *Bam*HI–*Bst* XI–*Eco*RI–*Hin*dIII (GGATTCCAGTGTGGTG-GTAGGGAATTCAAGCTT)–stuffer fragment (1000 bp)–*Not* I–*Bst* XI–*Sal* I (CTCGAGCGGCCGCCAGCACAGTG-

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Abbreviations: IL, interleukin; IL-3Rα, α subunit of the IL-3 receptor; SV40, simian virus 40; ori, origin; T, tumor; GM-CSF, granulocyte/macrophage colony-stimulating factor; m, murine; h, human; FCS, fetal calf serum; FACS, fluorescence-activated cell sorter; LTR, long terminal repeat; TK, thymidine kinase.

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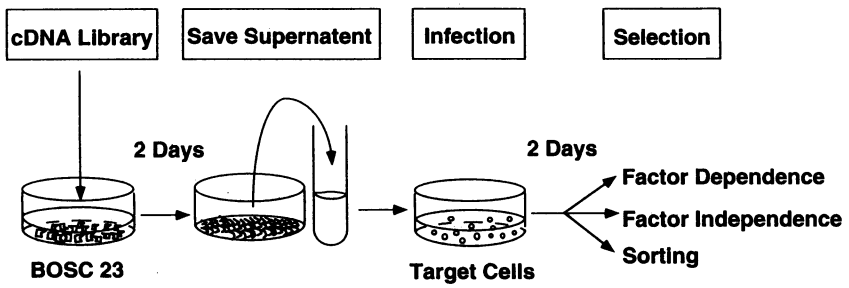

FIG. 1. Overall procedure of expression cloning using retrovirus cDNA libraries.

GTCGAC). This modified vector was designated pBabeX. To construct a retrovirus vector carrying a cDNA for the hIL-3Rα, the cDNA insert was excised from the pDUK-1 (9) and inserted into the *Sna*BI site of pBabe-neo vector (designated pBabe-neo-DUK) or the *Eco*RI–*Not* I site of the pBabeX vector (designated pBabeX-DUK). The retroviral construct of pMTneo carrying a cDNA encoding the receptor for murine ecotropic viruses (15) was a gift from J. D. Norton. MFG-lacZ and pZipNeoSV(X)-1 (16) were kindly provided by R. C. Mulligan.

cDNA Library Construction. Poly(A)$^+$ RNA was prepared from TF-1 cells and a mixture of human T-cell clones by using FastTrack (Invitrogen). Unidirectional cDNA libraries were constructed by using a cDNA synthesis kit and a directional cloning tool box (Pharmacia) by the manufacturer's suggestions, with some modifications. Double-stranded cDNA were synthesized from 1–2 μg of mRNA and size-fractionated through a 0.9% agarose gel, and cDNAs of 1.0–7.0 kbp were cloned into the *Eco*RI–*Not* I site of the pBabeX vector (200 ng)

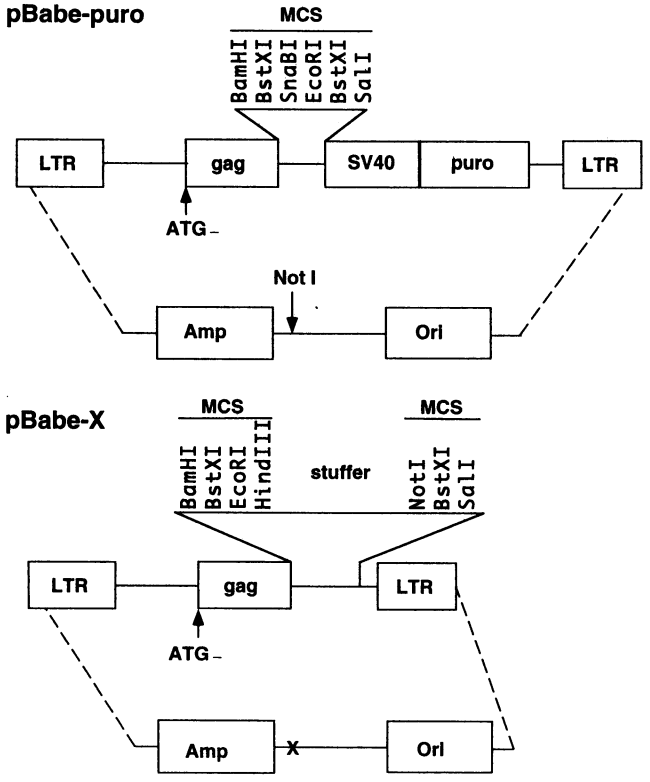

FIG. 2. Schematic structures of pBabe-puro and pBabe-X vectors. The pBabe-puro vector (*Upper*) was modified by disrupting a *Not* I site (indicated by X in *Lower*), deleting the drug selection marker, and adding a new multicloning site (MCS) containing a 1-kbp stuffer. The resulting vector is designating pBabe-X (*Lower*). LTR, long terminal repeat; SV40, SV40 early promoter; Amp, ampicillin resistance gene; Ori, replication origin of pUC.

by using the Takara Shuzo (Kyoto) ligation kit. The ligated DNA was transformed into Max Efficiency electrocompetent cells (GIBCO/BRL) according to the manufacturer's recommendation. The cDNA libraries contained 0.5–2 $\times$ 10^6 clones, >90% of which had cDNA insertions. The average size of the insertions was 1.5 kbp.

Production of Retrovirus Stock. BOSC23 cells (2 $\times$ 10^6 cells) were seeded onto 60-mm dishes 1 day before the transfection. Transfection by LipofectAmine reagent was performed according to the manufacturer's protocol. Cells were cultured for 48 hr; the retroviral supernatant was then used for infection of target cells. The estimated titers of the retroviruses were 1–3 $\times$ 10^6 colony-forming units/ml based on G418-resistant colony formation of the infected NIH 3T3 cells.

Infection and Transient Expression of Recombinant Retroviruses. To optimize the conditions of transfection/infection and to monitor the virus titer, we used test constructs of a retroviral vector carrying the hIL-3Rα cDNA. For infection of NIH 3T3 cells, 2 $\times$ 10^5 cells were seeded into 60-mm plates the night prior to infection and incubated with 1.5 ml of virus stock for 6–8 hr in the presence of Polybrene (8 μg/ml). Then, 1.5 ml of fresh DMEM/10% FCS containing Polybrene (8 μg/ml) was added to the culture and the incubation was continued. The medium was changed to fresh DMEM/10% FCS 24 hr after the beginning of the infection. After another 24 hr, the cells were removed from the plates and stained with anti-IL-3Rα antibody (N3A) (13).

For infections of hematopoietic cell lines, 1 $\times$ 10^6 cells were incubated with 3 ml of virus stock containing Polybrene (10 μg/ml) and the cytokine necessary to support cell growth (mIL-3 or hGM-CSF). The rest of the procedure for infection is the same as that for NIH 3T3 cells described above. cDNA library viruses were generated and infected into cells in general as above.

Sequencing of the Integrated Retroviruses. To sequence integrated cDNAs, a small amount (50 ng) of genomic DNA isolated from each clone was subjected to PCR. cDNA segments were amplified by using upstream and downstream retroviral vector primers (5′-CAGCCCTCACTCCTTCTC-3′ and 5′-GGTGGGGTCTTTCATTCC-3′). The PCR was run for 35 cycles (1 min at 94°C, 2 min at 56°C, and 3 min at 72°C) by using *Taq* polymerase (Perkin–Elmer/Cetus). The resulting PCR fragment was purified and sequenced using the Taq DyeDeoxy Terminator cycle sequencing kit (Applied Biosystems) carried out on an Applied Biosystems model 373A sequencer.

RESULTS

Infection Efficiency of Test Constructs. NIH 3T3 cells were infected with viruses carrying hIL-3Rα cDNA with and without the neomycin-selectable gene. As shown in Fig. 3 *A* and *B*, viruses generated from pBabeX-DUK can infect virtually 100% of NIH 3T3 cells. This is more efficient than the pBabe-neo-DUK viruses, which gave 70% infection of NIH 3T3 cells and a titer of 3 $\times$ 10^6 colony-forming units/ml based

Table 1. Two model experiments

	Exp. 1	Exp. 2
Complexity of cDNA library	0.6×10^6	1×10^6
No. of virus particles used for infection*	$>3 \times 10^7$	$>3 \times 10^7$
No. of cells used for infection	2×10^6	3×10^6
No. of cells used for selection	1×10^7	1.5×10^7
Predicted no. of infected cells†	1×10^6	2.2×10^6
Predicted frequency of target cDNA‡	1 in 10^4	1 in 1.5×10^5
Predicted no. of $CD2^+$ cells§	100	NA
Total no. of sorted cells	2000	NA
Predicted % of $CD2^+$ cells§	5	NA
Actual % of $CD2^+$ cells§	8	NA
Predicted no. of hIL-3Rα^+ Ba/F3 cells‖	NA	15
Actual no. of hIL-3Rα^+ Ba/F3 cells‖	NA	13

NA, not available.
*As measured on NIH 3T3 cells by neomycin resistance.
†Based on infection efficiency of hIL-3Rα of sib cells at the time of cDNA library infection.
‡Estimated by the Southern blot analysis of subdivided pools of the library.
§At the time of or after the first sorting.
‖When the selection in the presence of hIL-3 starts.

on the number of G418-resistant colonies. Retroviruses derived from pBabeX-DUK were infected into an mIL-3-dependent cell lines Ba/F3, and the hIL-3Rα/hGM-CSF-dependent TF-1 cell line that was transduced with the plasmid (pMTneo) encoding the receptor for murine ecotropic viruses (15) (designated TF-1/ecoVR). Infection efficiency as evaluated by FACS analysis (Fig. 3 *C* and *D*) was as follows: 15.8% for Ba/F3 cells and 37% for the TF-1/ecoVR.

Screening of Viral cDNA Libraries. To test whether representative transfer of mixed cDNA populations could be accomplished, we set up a reconstruction/competition experiment with a *lacZ*-encoding virus vector and a neomycin-resistance gene-encoding virus vector. The results indicated that we could readily detect a neomycin-resistant virus at a ratio of 1 in 10^7 after transfection of BOSC23 cells with a mixture of MFG-lacZ and pZipNeoSV(X)-1 (M. Rothenberg and G.P.N., unpublished results). We therefore proceeded with cDNA library construction and generation of retrovirus representing an endogenous mRNA mixture.

Two experiments were designed to test our retroviral cDNA expression cloning system (summarized in Table 1). In the first experiment, we attempted to isolate a cDNA for CD2 antigen (4) from a retroviral cDNA library by using flow cytometry to select cells transduced with the CD2 cDNA. A cDNA library was constructed from a mixture of several human T-cell clones that expressed high levels of CD2 antigen as determined by FACS analysis. Ba/F3 cells (2×10^6 cells) were infected with 9 ml of virus stock produced by the human T-cell cDNA library. Two days after infection, the infected Ba/F3 cells were collected (1×10^7 cells), stained with fluorescein isothiocyanate-conjugated anti-CD2 antibody (Fig. 4 *A* and *B*), and subjected to cell sorting. The sorted cells (2000 cells) were expanded for 5 days in a bulk culture and reanalyzed by FACS. Eight percent of the sorted cells were positive for human CD2 (Fig. 4*C*). These cells were subjected to a second round of sorting and the sorted cells were again expanded in bulk culture. These cells were nearly 100% positive for CD2 staining (Fig. 4*D*). The CD2-positive Ba/F3 cells were then subjected to single-cell sorting, and 10 subclones were expanded for further analysis. Genomic DNAs extracted from these subclones gave rise to a common 1.6-kbp band after PCR amplification using viral vector primers. There was an additional band in one clone (Fig. 6*A*). Two of the 1.6-kbp PCR products

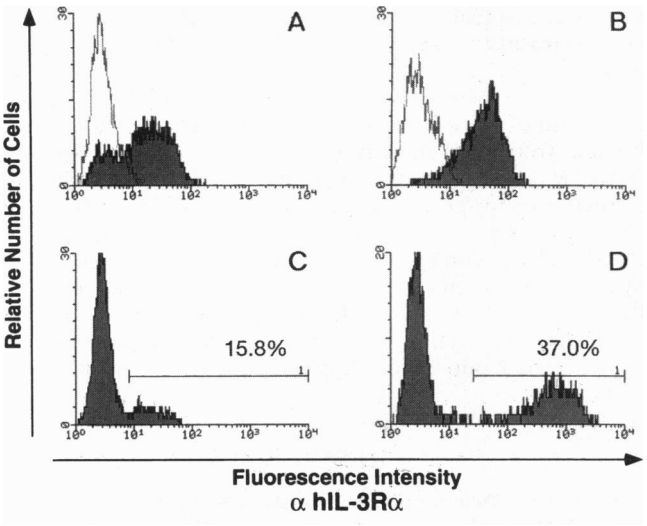

FIG. 3. Infection efficiency of pBabe retroviruses. NIH 3T3 cells were infected with the pBabe-neo-DUK virus (*A*) or the pBabe-X-DUK virus (*B*) and tested for the expression of hIL-3Rα by fluorescence-activated cell sorter (FACS) analysis. Ba/F3 cells (*C*) and TF-1/ecoVR (*D*) were infected with the pBabe-X-DUK virus and tested for the expression of hIL-3Rα by FACS analysis.

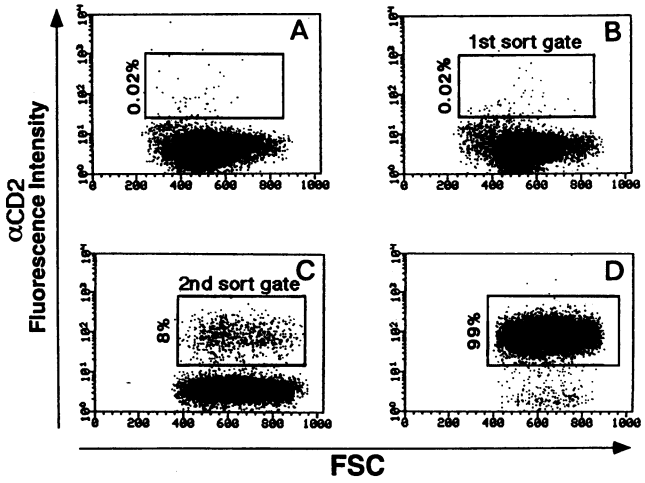

FIG. 4. Experiment 1: Expression cloning of a surface molecule CD2 from a retroviral cDNA library. CD2 expression of parental Ba/F3 cells and the Ba/F3 cells infected with viruses derived from a human T-cell cDNA library. (*A*) Ba/F3 cells before infection. (*B*) Ba/F3 cells after the infection. (*C*) Ba/F3 cells after the first sorting. (*D*) Ba/F3 cells after the second sorting. Boxed regions indicate CD2-positive cells; the cells in the boxes were sorted in *B* and *C*. The cells in the boxed region in *A* are background staining.

were sequenced and were confirmed to be identical to the human CD2 cDNA (4).

The most important advantage of the expression cloning method using retrovirus system is that one might use any functional assay for selection. In the second experiment, we attempted to isolate a hIL-3Rα cDNA by using hIL-3-dependent growth of Ba/F3 cells as a selection method. A cDNA library was constructed from a hIL-3/hGM-CSF-dependent TF-1 cell line. Ba/F3 cells (3×10^6 cells) were infected with 9 ml of virus stock produced from the TF-1 cDNA library, and the infected Ba/F3 cells had proliferated by 5-fold in 2 days after the infection (Table 1). As shown in Table 1, we supposed that 2.2×10^6 Ba/F3 cells harbored cDNA library viruses at the time of starting selection and that 15 cells of the 2.2×10^6 Ba/F3 cells harbored the cDNA virus encoding the hIL-3Rα. To functionally select those Ba/F3 cells transduced with the hIL-3Rα virus, the infected cells were selected in the presence of hIL-3 (100 ng/ml) in 24-well plates based on the observations (13) that Ba/F3 cells expressing hIL-3Rα can be maintained in the presence of hIL-3, but that parental Ba/F3 cells do not respond to hIL-3. Growing cells were observed in 13 out of 48 wells after 1 week in culture, and all these clones showed hIL-3-dependent growth. The expression of hIL-3Rα was examined by FACS analysis after expanding the growing cells in the presence of mIL-3 for 1 week, and expression of hIL-3Rα was detected in all clones (Fig. 5 *A–M*). Bimodal expression of hIL-3Rα was detected in clones 7 and 13 (Fig. 5 *G* and *M*); one of two peaks seemed to be negative for the expression of hIL-3Rα in both clones. After culturing these clones in the presence of mIL-3 for another week, the expression of hIL-3Rα remained the same in clone 13 (Fig. 5*O*) but disappeared in clone 7 (Fig. 5*N*). The positive peak of clone 13 was strongly enhanced by culturing this clone in the medium containing hIL-3 (Fig. 5*P*). Genomic DNAs extracted from clones 2, 7, and 13 and a subclone of clone 13 (clone 13-18) were subjected to PCR analysis with the virus vector primers.

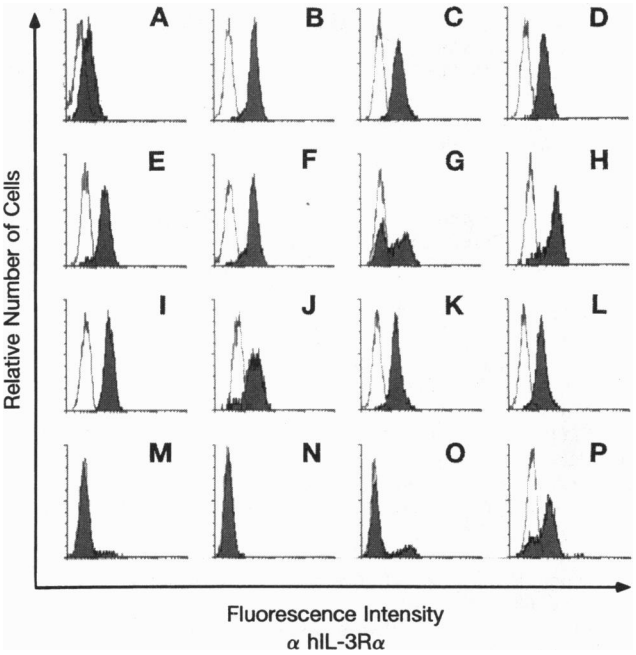

FIG. 5. Experiment 2: Phenotype complementation cloning of hIL-3Rα. IL-3Rα expression of the Ba/F3 subclones (infected with viruses derived from the TF-1 cDNA library) was selected in the presence of hIL-3 (100 ng/ml) and examined by FACS analysis with the monoclonal antibody N3A. (*A–M*) Clones 1–13. (*N*) Clone 7 after 2 weeks culture in the presence of mIL-3. (*O*) Clone 13 after 2 weeks culture in the presence of mIL-3 (clone 13L). (*P*) Clone 13L after 2 weeks culture in the presence of hIL-3.

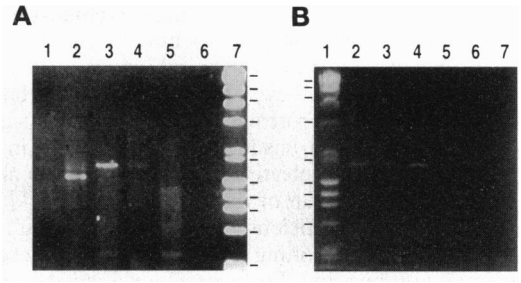

FIG. 6. Retroviral inserts amplified by retroviral vector primers. Genomic DNAs were extracted from CD2-positive and hIL-3R-positive Ba/F3 infectants. cDNA inserts in these DNA were amplified by PCRs using 5′ and 3′ retroviral vector primers; the PCR products were subjected to electrophoresis through 0.8% agarose gel. (*A*) Lanes: 1, clone 1; 2, clone 3; 3, clone 8; 4, clone 9; 5, Ba/F3 cells; 6, PCR-negative control without any template DNA; 7, λ*Hin*dIII and ψX174-*Hae* III DNA markers (tick marks indicate 23, 9.6, 6.4, 4.3, 2.2, 2.0, 1.4, 1.0, 0.8, 0.6, and 0.3 kbp from top to bottom). (*B*) Lanes: 1, λ*Hin*dIII and ψX174-*Hae* III DNA markers; 2, clone 2; 3, clone 7; 4, clone 13; 5, clones 13-18; 6, Ba/F3 cells; 7, PCR-negative control without any template DNA. Tick marks are as in *A*.

Although clones 7 and 13-18 were negative for the expression of hIL-3Rα at the time of DNA extraction (Fig. 5*N* and data not shown), all these clones gave rise to a common 1.8-kbp band (Fig. 6*B*). Two of those PCR fragments were sequenced and confirmed to be derived from hIL-3Rα cDNA. These data indicated that some of the integrated viruses became transcriptionally silent in the absence of positive selection.

DISCUSSION

We have established a high-efficiency cDNA expression cloning system that utilizes retroviruses as a gene transfer tool and, thus, have overcome two major limitations of the COS-cell expression cloning system (i.e., restriction of target cells and selection methods). In this communication, we present two experiments that demonstrate the efficiency and convenience of our retroviral expression cloning system.

In the first experiment, we have shown that cDNAs for surface markers such as CD2 could be readily cloned by the retroviral expression cloning system. In cloning cDNAs for surface molecules, retrovirus-mediated cloning has two advantages over the conventional COS-cell expression cloning systems. First, in conventional expression cloning by panning (4, 5) or sorting (6), the plasmid DNA recovered from selected COS cells needs to be transfected repeatedly into COS cells. In contrast, with retrovirus expression cloning, a single infection can give rise to stable expression of an introduced gene. Therefore, once infected, the cells expressing a desired surface marker can be selected simply by enriching them by multiple FACS sortings or panning. More importantly, availability of a wider range of target cells in the retrovirus expression system should make it possible to clone surface molecule genes that could not be cloned in COS cells because of the presence of cross-reactive molecules or the absence of auxillary molecules on COS cells or other unknown factors such as post-translational modifications.

In the second experiment, we applied a functional assay (factor-dependency) that cannot be used in COS-cell expression cloning. The frequency of hIL-3Rα cDNA in the library was determined by the Southern blot analysis of the subdivided pools of the cDNA library and was estimated to be 1 in 1.5×10^5 (9). Thus, a cDNA present at this low frequency can be cloned by the retrovirus expression system at a reasonable frequency (Table 1). We noted in this experiment that in some clones (2 out of 13 clones in this case), the expression of hIL-3Rα decreased or disappeared during the culture of these clones in the presence of mIL-3. The decreased expression of

hIL-3Rα was partly reverted by culturing the clone 13 in the presence of hIL-3, indicating that selective pressure was required for stable expression of retroviral inserts in this clone (Fig. 5). However, we believe that this will not hamper expression cloning using retrovirus infection, because the expression of retrovirally transduced genes is stable in most clones and because some selective pressure should be able to sustain the stable expression of the gene of interest.

Since retroviruses can efficiently infect a variety of cell lines, the retrovirus expression cloning system now makes interesting selection strategies possible. For example, we may be able to clone signal transduction molecules by complementation of signal transduction pathways, artificially disrupted by introduced mutations. In addition to cloning genes, this system is also useful in structure–function analysis of known proteins. Namely, random mutations can be introduced into a particular molecule and the effect of this mutagenesis on its function can be assessed by introducing the mutated molecules into specific target cells via retrovirus infection.

Two other groups have recently published expression cloning systems using retrovirus cDNA libraries (17, 18). As a cDNA library source, Wong *et al.* (18) utilized a packaging cell line that had been stably transfected with retrovirus cDNA libraries to generate polyclonal pools of producer cells. Rayner and Gonda (17) transiently transfected cDNA libraries into amphotropic packaging cells; subsequently, ecotropic packaging cells were stably infected with the generated amphotropic viruses, selected by drug resistance, and used as a cDNA library source. A major limitation of the approach by Wong *et al.* (18) is that the use of stable transfection of packaging cells to generate viruses severely limits the representation of the library as infectious retroviruses, since the efficiency of stable transfection is orders of magnitude below the efficiency of transient transfection. Although the latter group utilized retrovirus infection instead of transient transfection to achieve more efficient transduction of cDNAs into packaging cells, the titier of the amphotropic retroviruses used for the infection was very low ($1–3 \times 10^4$ viruses per ml) and, therefore, it would be difficult to cover the complexity of large cDNA libraries. In addition, in both studies, drug selection (neomycin resistance) was used to establish packaging cells expressing cDNA libraries, and this may cause changes in the frequency of individual cDNAs in the library during the selection and long-term culture of stable packaging cell lines. Moreover, these procedures are time-consuming, and strategies requiring continuous maintenance of the packaging cells often result in decreased titer of retroviruses due to retroviral expression shutdown. In the past, to establish a retrovirus expression cloning system, Murphy and Efstratiadis (19) utilized a compound λ-based vector, from which retrovirus DNAs were released by homologous recombination of two LTRs in the vector. To rescue a thymidine kinase (TK) cDNA, they infected cDNA retroviruses into TK^- NIH 3T3 cells by cocultivation with the packaging cell line. They then fused the cells with the desired phenotype (in this case TK^+) to WOP cells, in which polyoma origin-bearing plasmids can be amplified, and recovered the retrovirus constructs from Hirt supernatants. Although their strategy in making retroviruses and recovering retrovirus cDNA was elegant, the titer of the retroviruses was not described in the paper, and they only used TK^- NIH 3T3 cells as target cells.

In our transient approach, we maintain the cDNA library in a stable DNA form and are able to make either ecotropic or amphotropic viruses simply by transfecting the same DNA into different packaging lines. Our method does not require any drug selection marker in the vector. As demonstrated in Fig. 3, and also as has been suggested by other researchers (21), inclusion of additional DNA such as a drug selection gene in retroviral vectors can lower the titer of retroviruses due to packaging size constraints, promoter interference, or other structural complications. By using the transient packaging system and thus avoiding the usage of drug selection markers, we could readily obtain high-titer retroviruses that can infect hemopoietic cells efficiently.

The power of complementation cloning, long appreciated in bacterial and yeast genetic systems, may now be more fully accessed for mammalian cells by the retroviral approaches we described herein. Complementation analysis has provided several important genes to date, such as a recombination activating gene *RAG1* (20). We expect that the retroviral cDNA library transfer approach offers numerous advantages to those interested in complementation cloning in mammalian cells.

Note Added in Proof. Whitehead *et al.* (22) have recently published expression cloning of oncogenes based on retrovirus-mediated cDNA library transfer into NIH 3T3 cells.

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