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High-Efficiency Identification of Genes by Functional Analysis from a Retroviral cDNA Expression Library

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Retroviral gene transfer efficiently delivers genes of interest stably into target cells, and expression cDNA cloning has been shown to be highly successful. Considering these two advantages, we now report a method by which one can identify genes stimulating cell growth through functional analysis. The first step requires the construction of a retroviral cDNA expression library and the optimization of transfection of vector DNA into virus packaging cells. The second step involves the cocultivation of target cells with libraries of retrovirus-producing cells, resulting in the amplification of target cells transduced with a gene(s) stimulating cell growth. Under standardized conditions of transfection, we detected an average of 4,000 independent clones per dish, among which expression of a retroviral β-galactosidase gene at an abundance of 0.2% could be detected. Next, we demonstrated the augmentation of the sensitivity of the assay by retroviral infection and functional analysis. We did this by cocultivating factor-dependent (FD) cells with dishes of GP/E cells transfected with plasmids containing various molar ratios of pN2-IL3 DNA and retroviral library cDNA and by determining the highest dilution of pN2-IL3 which still resulted in the conversion of FD cells to factor independence. The retroviral interleukin-3 gene at an abundance as low as 0.001% could be detected. Indeed, we were able to detect from FD cells the development of factor-independent colonies with different phenotypes after retroviral transfer of cDNAs from an immortalized hemopoietic stem cell line. Thus, the combination of a standardized high-efficiency DNA transfection and retrovirus-mediated gene transfer should facilitate the identification of genes capable of conferring to target FD cells a detectable new function or phenotype. By scaling up the size of the experiment realistically during screening, the assay can detect cDNA at an abundance of lower than 0.0001%.

Among the different methods of gene transfer, the use of retrovirus vectors has been shown to be highly desirable. Foreign DNA of up to 10 kb may be efficiently delivered and stably incorporated into target cells (2, 10, 14–17, 21, 28, 34, 39, 58, 59). Packaging cell lines which allow the generation of helper-free recombinant retrovirus vectors at titers as high as 10^7 infectious particles per ml are available. The system itself is well characterized and considered to be relatively safe for use in gene therapy, despite the infrequent occurrence of insertional mutagenesis.

Expression cloning of genes without much knowledge of the gene products has been reported by a number of investigators (45). This is usually achieved by assays in which specific biological activities can be measured. Size-selected mRNAs are then converted into cDNAs in plasmid vectors, which are then transfected into COS cells for high levels of transient expression of the gene products (1). In this way, cDNAs of interleukin-3 (IL-3) (69), granulocyte-macrophage colony-stimulating factor (GM-CSF) (35), IL-2 (68), B-cell stimulatory factor 1 (36), IL-4 (33), receptor of human GM-CSF (19), receptor of granulocyte colony-stimulating factor (18), and IL-5 (60) have been isolated.

Hemopoiesis is regulated by a large family of molecules known as interleukins, cytokines, lymphokines, or hemopoietic growth factors. Many of these molecules are pleiotropic and redundant in function (49). Because of the biological properties of these molecules, several cell lines whose growth and proliferation were each initially established to be dependent on a particular known growth factor were subsequently found to be responsive to several other factors. For example, FDC-P1 and 32D cells have been shown to respond to IL-3 (6, 7, 13, 65), GM-CSF (26, 30, 31), macrophage colony-stimulating factor (55), granulocyte colony-stimulating factor (61), IL-2 (37, 48), and erythropoietin (44). By introduction and expression of a number of receptor genes into these cells, a growth response of FDC-P1 or 32D to the corresponding ligands is observed. These molecules are diverse in that they stimulate growth through different signal transduction pathways. They include colony-stimulating factor-1 (27), insulin-like growth factor-1 (42), IL-5 (60), erythropoietin (52), fibroblast growth factor (38), epidermal growth factor (51), and platelet-derived growth factor (41). Transduction into FDC-P1 and 32D cells with genes such as *myc* (3, 12, 53), *myb* (20), *myb-ets* (57), c-*cbl* (32), v-*fms* (25), v-*fps* (43), c-*erb-2* (67), *abl* (8, 9, 11, 24, 29, 47, 50), and *ras* (4, 54) also results in cellular proliferation without an exogenous supply of the original growth factor. Thus, it is clear that multiple signal transduction pathways are operative in these cells. It is therefore conceivable that FDC-P1 cell proliferation can be used as a functional assay to screen for novel factor(s) capable of replacing the original growth factor requirement.

Recently, we established an immortalized hemopoietic cell line, BL3, that retains certain properties of hemopoietic stem cells (66). BL3 cells were derived from a recipient of mouse fetal liver stem cells transduced with a retrovirus vector (64, 65) and were non-tumorigenic when injected into nude mice. We hypothesized that a single mutational event had taken place in BL3 cells, resulting in the activation of an endogenous gene whose expression led to stimulation of BL3 cell growth

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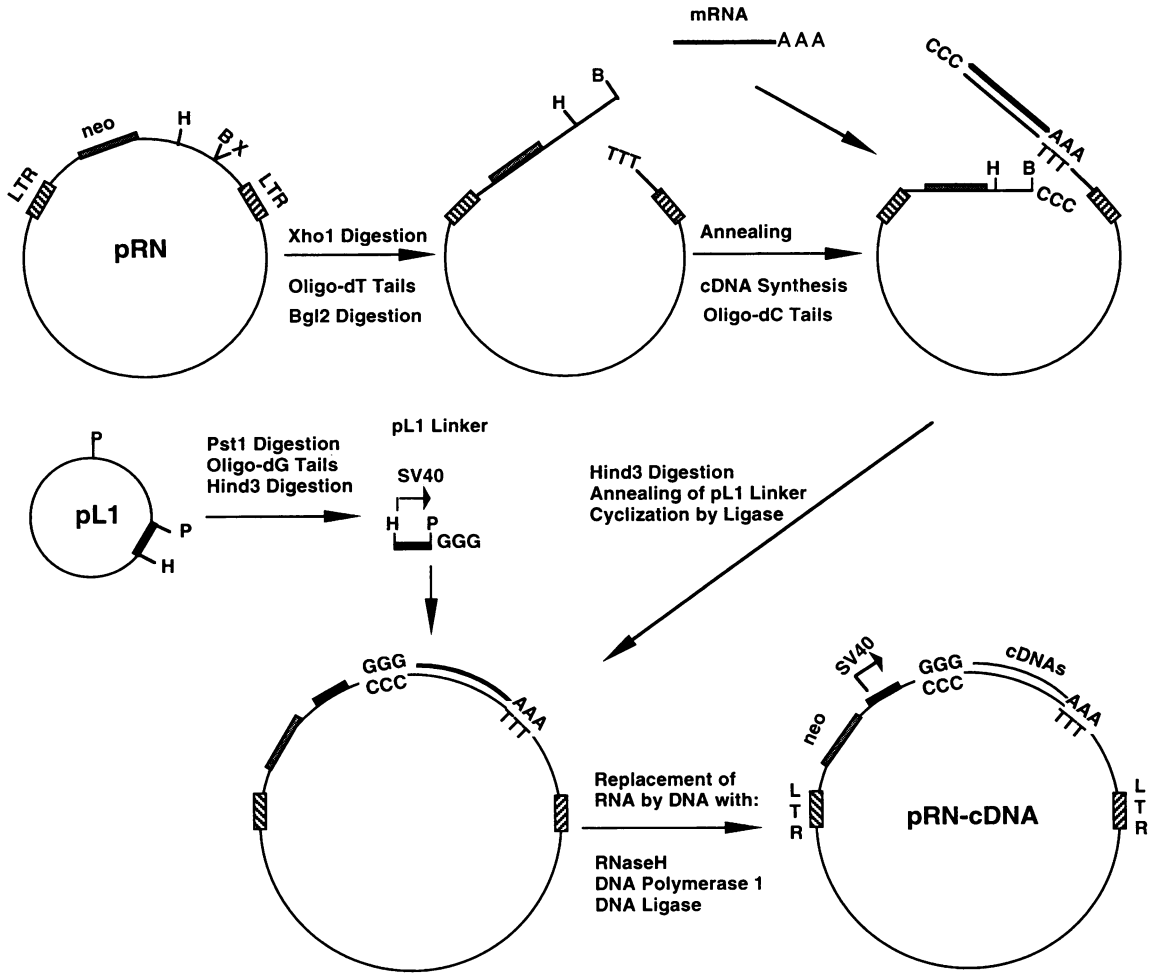

FIG. 1. Construction of pRN-BL3 retroviral cDNA library. LTR, long terminal repeat; SV40, simian virus 40; neo, neomycin.

and immortalization. At that time, we decided to establish a method to identify this gene without much knowledge of its gene product. It is obvious that the gene of interest can encode a secretory growth factor or a cytoplasmic or nuclear molecule. Despite its unknown nature, this molecule should participate in the signal transduction pathway affecting cell growth.

In this report, we establish a method with which a growth-stimulating gene can be detected from a library of plasmid DNA consisting of at least 100,000 independent clones. With this efficiency, it becomes feasible to screen for even regulatory genes, whose mRNAs are usually present in low abundance. This method entails the construction of a retrovirus vector cDNA library (pRN), transfection of the pRN library plasmid DNA into packaging cells, cocultivation of FDC-P1 with packaging cells to produce a library of recombinant retroviruses, and factor-independent (FI) growth of retrovirus-transduced FDC cells as an assay to screen for the gene product with growth-stimulating activity.

(Part of this work was presented at the 1993 annual meeting of the American Society of Hematology in St. Louis, Mo.)

MATERIALS AND METHODS

Construction of retroviral vector pRN. To accommodate future cDNA inserts, we constructed a small retroviral vector plasmid, pRNT, by ligating the 4-kb *Kpn*I fragment from pXT1 and the 3.8-kb *Kpn*I fragment from pUC-ABL. *Bam*HI and *Hin*dIII sites located outside the proviral sequence of pRNT were digested and blunt-end ligated, respectively. To create a *Hin*dIII cloning site, a linker DNA, CAAGCTTG, was inserted into the *Sal*I site blunted by Klenow, generating the 7.8-kb pRN plasmid shown in Fig. 1.

Construction of retroviral vector primer. To prepare retroviral vector primer DNA, we digested pRN with *Xho*I. This was followed by a Klenow fill-in reaction. Next, we performed oligo(dT) tailing of pRN/*Xho*I DNA (46). A small-scale reaction produced an estimate that the addition of 40 to 60 dT residues per tail would take 30 min. A large-scale reaction based on this estimate was then set up. To remove the 5′ oligo(dT) end, we digested the DNA with *Bgl*II. A small-scale *Bgl*II digestion of pRN/*Xho*I/dT was done in a 20-μl reaction volume, and the minimal incubation time and the amount of enzyme required for complete digestion of DNA were determined. This was followed by a large-scale *Bgl*II digestion of DNA, which was then purified by sucrose gradient centrifugation.

To select poly(dT)-vector primer DNA, a 5-ml pipette column containing 0.16 g of oligo(dA)-cellulose was equilibrated with 1× loading buffer (1 M NaCl, 10 mM Tris-Cl, 1 mM EDTA [pH 7.5]). pRN/*Xho*I/dT/*Bgl*II DNA was loaded

onto the column twice, eluted with diethyl pyrocarbonate (DEPC)-treated H_2O, and collected in 0.5-ml fractions; those fractions containing DNA were combined, purified, and stored in Tris-EDTA until used.

Purification of poly(A) RNA. BL3 cells (1.2×10^9) were harvested and lysed in GTC solution, and poly(A) RNA was isolated as described previously (46). Oligo(dT)-cellulose (0.1 g) was suspended in 10 ml of 0.1 N NaOH and packed in a 5-ml pipette used as a column. The column was washed with 10 ml of DEPC-H_2O and then 7 ml of 1× binding buffer (0.5 M NaCl, 10 mM Tris-Cl [pH 7.5], 1 mM EDTA [pH 7.0]). Total RNA (1.8 mg) in a 300-μl solution was mixed with 350 μl of DEPC-H_2O and 650 μl of 2× binding buffer, heated at 65°C for 5 min, and then loaded onto the column. The eluate was collected, heated, and reloaded. The column was washed with 7 ml of 1× binding buffer. Poly(A) RNA was eluted with 3 ml of DEPC-H_2O. The eluate was mixed with an equal volume of 2× binding buffer, heated, and reapplied to the column equilibrated with 1× binding buffer. DEPC-H_2O eluate was collected. The amount of poly(A) RNA was determined, and the sample was stored at −70°C until used.

cDNA cloning. For first-strand synthesis, we compared the efficiency of cDNA synthesis in small-scale reactions by using (i) no primer, (ii) oligo(dT) primer, or (iii) retroviral vector primer. Reactions were carried out under conditions similar to those described previously (46). Results indicated that in the absence of a primer, there was no incorporation of the radioactive isotope and that an incorporation of 20 and 14% occurred in reactions containing oligo(dT) and retroviral vector primers, respectively. Following that, a large-scale cDNA synthesis was performed with a 45-μl reaction. At the end of the reaction, cDNA synthesis was monitored as in the small-scale reaction.

Purified cDNA was subjected to dC tailing, a half-scale pilot reaction containing 11 μl of DNA from the previous step, 4 μl of 5× TdT buffer (Boehringer Mannheim Biochemicals [BMB]), 1.5 μl of 10 mM $CoCl_2$, and 1 μl each of 1 mM dithiothreitol, 0.3 μg of poly(A) per μl, and 1.2 mM [^{32}P]dCTP (5 μl of 2 mM dCTP mixed with 3.3 μl of [^{32}P]dCTP), and was incubated at 37°C for 15 min. A 0.6-μl aliquot was removed and precipitated in 10% trichloroacetic acid as described earlier. dC tailing was initiated by adding 1 μl of 25-U/μl TdT (BMB) and incubating at 37°C for 10 min. Another 0.6-μl aliquot was taken and precipitated in 10% trichloroacetic acid. The radioactivity of each aliquot was measured for a calculation of the number of dC residues per end. dC-tailed DNA was then purified and dissolved in 26 μl of H_2O. Three microliters of 10 × buffer B (BMB) and 1 μl of *Hin*dIII (10 U/μl) (BMB) were added. The mixture was incubated at 37°C for 60 min and ended by adding stop buffer. The sample was then purified and dissolved in 12 μl of H_2O.

To circularize the mRNA-cDNA-vector hybrid molecule, we performed the following reactions. Six microliters of *Hin*dIII-digested DNA from the last reaction, 2 μl of 0.05-pmol/μl oligo(dG)-tailed linker DNA (Pharmacia), and 2 μl of 5× hybridization buffer (50 mM Tris-Cl [pH 7.5], 5 mM EDTA, 500 mM NaCl) were mixed, incubated at 65°C for 5 min and 43°C for 30 min, and put on ice. Eighteen microliters of 5× ligase buffer [100 mM Tris-Cl (pH 7.5), 20 mM $MgCl_2$, 50 mM $(NH_4)_2SO_4$, 500 mM KCl, 250 μg of bovine serum albumin per ml], 70 μl of H_2O, and 1 μl of 10 mM β-NAD were added to the reaction tube. The mixture was incubated on ice for 10 min, and then 3 μl of *Escherichia coli* DNA ligase (2 U/μl) (BMB) was added. The reaction tube was incubated at 12°C overnight. Next, the following were added to the tube: 4 μl of 1 mM deoxynucleoside triphosphate, 0.5 μl of 10 mM β-NAD, 3 μl of *E. coli* DNA ligase, 2 μl of DNA polymerase (BMB), and 2 μl of RNase H (1 U/μl) (BMB). The mixture was incubated at 12°C for 1 h and then at room temperature for 1 h. This product was stored at −20°C until used. Competent DH5α cells were made according to Hanahan's method (23) with efficiencies of 1×10^8 to 10×10^8 colonies per μg of pBR322. The ligation mixture was divided into aliquots of 2 to 4 μl, each of which was used to transform 0.2 μl of competent cells (23). Tubes were pooled, and six sublibraries were constructed, with sizes ranging from 2.3×10^4 to 1.3×10^5 per sublibrary.

Cell culture. GP/E retroviral packaging cells (40) were obtained from Arthur Bank (Columbia University) and passaged in Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum. FDC-P1 cells were maintained in RPMI medium supplemented with 10% fetal bovine serum and 10% WEHI-conditioned medium. Coculture infection was performed in alpha medium supplemented with 10% fetal bovine serum (α10F), 10% WEHI-conditioned medium, and 5 μg of Polybrene per ml.

Transfection. GP/E cells were trypsinized, replated at 0.5×10^6 to 2×10^6 cells per 100-mm-diameter dish, and used the next day. Approximately 1 h prior to the addition of transfection reaction mixtures to cell cultures, dishes of GP/E were refed with 5 ml of fresh medium. Reaction mixtures were prepared by mixing up to 50 μg of plasmid DNA with 250 μl of 2× HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid)-buffered saline and water to a total volume of 500 μl. Thirty microliters of 2 M $CaCl_2$ was subsequently added dropwise for a final volume of 530 μl of reaction mixture per dish. Reaction mixtures were allowed to stand at room temperature for 0.5 h and were then added dropwise to the appropriate dishes. Dishes were incubated at 37°C for 6 to 12 h, at which time transfection was ended by replacement with fresh medium. Plasmid DNAs used included pJRgal (62), pLTK (5), pN2-IL3 (65), and pRN-BL3, a BL3 retroviral vector cDNA library. All vectors contained a neomycin resistance (Neo^r) gene, facilitating the selection of transfectants.

Assay for transfectants. Neo^r was assayed by G418 selection. One day after transfection, medium containing 1 mg of G418 per ml was added to cell cultures; it was replaced whenever it became acidic, usually within 4 to 5 days. Resistant colonies were counted after approximately 2 weeks. β-Galactosidase-positive (β-Gal$^+$) cells were assayed by X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) staining (62) 2 to 4 days after transfection. Genomic DNA samples from pN2-IL3- and pRN-BL3-infected FDC-P1 cells were subjected to Southern blot analysis with IL3- and neomycin-specific probes.

RESULTS

Experimental design. Figure 2 displays the structures of the parental pRN retrovirus vector and the pRN-cDNA vector. In the pRN vector, a thymidine kinase promoter is present downstream of the Neo^r gene, which is driven by the long terminal repeat promoter. In pRN-cDNA, the Neo^r gene is also driven by the long terminal repeat promoter, but the cDNA insert is transcribed off the simian virus 40 promoter derived from the pL1 plasmid (Fig. 1). This design is similar to that reported previously (6, 65).

Figure 3 displays a strategy for screening genes from a retroviral cDNA expression library on the basis of available biological assays. We constructed the pRN-BL3 retroviral library by incorporating the advantages of the Okayama-Berg system, which include high-frequency generation of full-length cDNA inserts and their directional cloning into vectors (46). In this study, mRNA from immortalized hemopoietic stem cells,

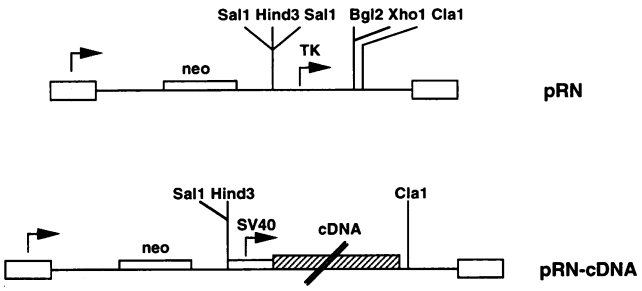

FIG. 2. Structures of the retroviral cDNA library vector, pRN-cDNA, and its parental vector, pRN. Note that the thymidine kinase (TK) promoter in pRN was replaced by a simian virus 40 (SV40) promoter in pRN-cDNA during the construction of the retroviral cDNA library. neo, neomycin.

BL3 (66), was reverse transcribed and cloned into the pRN vector. Six sublibraries were made. Plasmid DNA from each sublibrary was transfected into GP/E packaging cells; medium containing 1 mg of G418 per ml was added. Twelve days later, thousands of G418-resistant colonies per 100-mm-diameter dish were able to grow to near confluence. Factor-dependent (FD) cells were then added to the dishes for 2 to 3 days and assayed for FI growth.

Transfection standardization. Transfection was standardized by using the plasmid pJRgal under various conditions (Fig. 4). From six independent experiments under standardized conditions, an average of about 4,000 independent transfectants per dish was recorded when we used 50 μg of plasmid DNA for a 12-h exposure to calcium phosphate (Fig. 5).

To define the minimum relative abundance of mRNA required for successful cDNA expression cloning, various ratios of pJRgal and pLTK were transfected into GP/E cells.

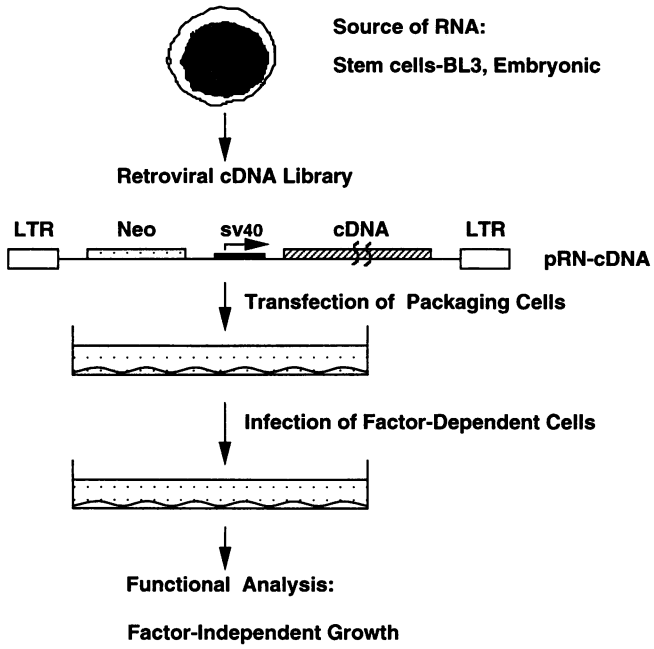

FIG. 3. Strategy of screening for genes by functional analysis with a retroviral cDNA expression library. LTR, long terminal repeat; Neo, neomycin; sv40, simian virus 40.

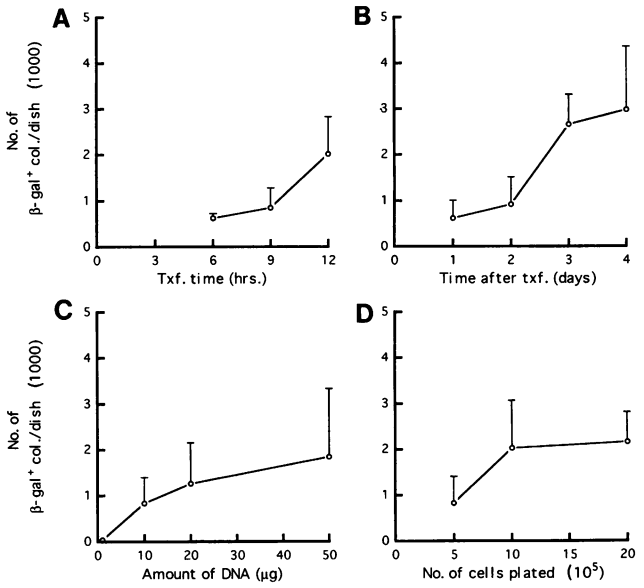

FIG. 4. Transfection standardization. GP/E cells were transfected with the plasmid pJRgal and X-Gal stained to assay for positive transfectants (see Materials and Methods). Unless otherwise specified, transfection was performed as follows: GP/E cells were prepared as described, plated at 10^6 cells per 100-mm-diameter dish, transfected with 20 μg of plasmid pJRgal per dish for 9 h, and then assayed by X-Gal staining 3 days posttransfection. Each panel shows the average of two experiments with duplicates at each point, except for panel C, which gives the average of three experiments. The standard deviation for each point is included. Results indicate that the optimal conditions consist of 10^6 cells per dish, transfection with 50 μg of plasmid DNA per dish for 12 h, and assay by X-Gal staining 4 days posttransfection. txf., transfection; col., colonies.

While both pJRgal and pLTK contain sequences conferring neomycin resistance, only pJRgal encodes the β-Gal gene. Results showed that while the number of Neo^r colonies remained relatively constant, the number of $\beta\text{-gal}^+$ cells varied directly with the ratio of pJRgal to pLTK (Fig. 6) and that it was detectable only when the ratio of pJRgal to pLTK was

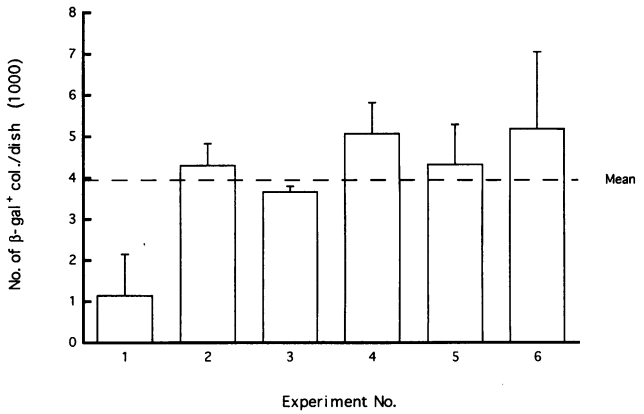

FIG. 5. Transfection under optimal conditions. Data are the results of six separate experiments; the median value is 3,948 transfectants per dish. col., colonies.

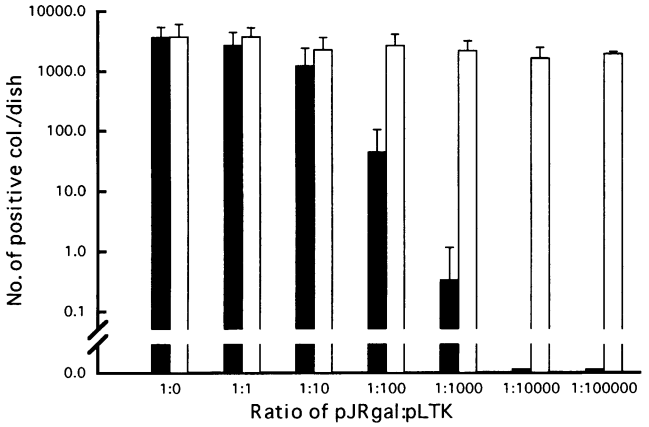

FIG. 6. Sensitivity of the assay based on X-Gal staining. GP/E cells were transfected with mixtures of plasmids pJRgal and pLTK; ratios of the plasmids varied from 1:0 to 1:100,000. In each experiment, three dishes of cells were transfected at each of the various ratios. Two dishes were assayed by X-Gal staining. The third dish was trypsinized immediately after transfection; cells were replated at dilutions of 1/10 and 1/100 and placed under G418 selection the next day. Counts were multiplied by the appropriate dilution factors, and the resulting numbers were averaged to yield an estimate of the number of colonies (col.) originally in the transfected dishes. Results are the averages of three separate experiments at each indicated ratio, except for the ratios 1:10 and 1:100,000, which were performed twice and once, respectively. Solid bars, β-Gal$^+$; open bars, Neo^r.

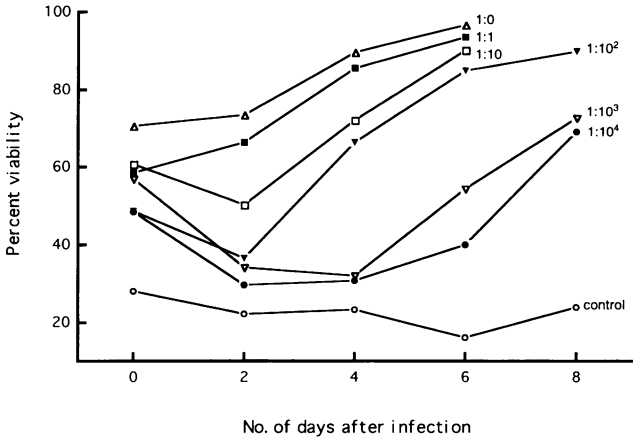

FIG. 7. Sensitivity of the assay based on IL3 biological function, FDC-P1 viability as a function of time and IL3 concentration. GP/E cells were transfected under optimal conditions with mixtures of plasmids pN2-IL3 and pRN-BL3 at ratios ranging from 1:0 to 1:10,000. Dishes were placed under G418 selection, and Neo^r colonies were allowed to expand until dishes approached confluence. At this point, 10^6 FDC-P1 cells were incubated in coculture with these virus-producing cells in 5 ml of α1OF medium with 10% WEHI-conditioned medium and 5 μg of Polybrene per ml. After 1 day of infection, another 5 ml of medium was added; infection was continued for 2 more days, after which FDC cells were collected, washed, and then cultured in the absence of WEHI-conditioned medium. Percent viability and total cell number were evaluated every 2 days by trypan blue exclusion. Individual curves correspond to FDC-P1 cells derived from coculture with GP/E cells transfected with the indicated ratios of pN2IL3 to pRN-BL3. Control FDC-P1 cells were cocultured with untransfected GP/E cells. Total cell number also increased with time, correlating in each case to percent viability (data not shown).

higher than 1:500, which was equivalent to an mRNA abundance of 0.2%.

The use of β-Gal might have caused the sensitivity in detecting a gene product capable of stimulating cell growth to be underestimated. Detection of this enzyme was done by X-Gal staining, a method in which a relatively high level of gene expression might be necessary. Indeed, we noticed that in dishes in which transfectants of pJRgal were used, β-Gal$^+$ colonies often contained no more than four positive cells that were richly stained with X-Gal after 4 days in culture. NIH/3T3-based cells used for transfection have a doubling rate of 12 to 16 h; therefore, each transfected cell should have undergone an average of six divisions normally. That less than four cells in a pJRgal colony stained positively with X-Gal indicated the level of β-Gal expression was a determining factor for detection by staining.

Enhanced detection based on biological assay. It is known that genes encoding growth factors or their receptors are usually expressed at low levels and are often detectable only by sensitive biological assays. Therefore, to investigate whether we could enhance the efficiency of this retroviral cDNA cloning, we employed the biological assays outlined in Fig. 3.

GP/E cells were transfected with mixtures of pN2-IL3 and pRN-BL3 at ratios of pN2-IL3 to pRN-BL3 ranging from 1:0 to 1:10,000. After infection for a period of 3 days, FDC-P1 cells were collected and cultured in the absence of WEHI-conditioned medium. Percent viability and total cell number were evaluated every 2 days. Results in Fig. 7 indicate that in all cases infected FDC-P1 cells were able to give rise to FI growth at rates significantly higher than that of the control culture. Furthermore, the growth rate was positively correlated with the amount of N2-IL3 used for transfection. Interestingly, although WEHI-conditioned medium was added during infection, the percentage of viability of FD cells was inversely correlated with the dilution of pN2-IL3. We interpret the data as indicating that during cocultivation, very high levels of metabolic activity of both virus-producing cells and target cells occurred. The amount of conditioned medium added was optimal for maintaining FD cells during passage, not for infection. Therefore, under this experimental condition, many FD cells not transduced with the N2-IL3 vector would not survive.

To further confirm the sensitivity of this assay, we determined by Southern blot analysis the highest N2-IL3 dilution at which FDC-P1 could still be transduced with the retroviral vector and result in Neo^r and FI growth. To examine whether multiple retroviral infections had taken place, we first did junction fragment analysis. Genomic DNA was digested with *Bgl*II, electrophoresed, and hybridized with either the IL3- or neomycin-specific probe. Figure 8a shows that FDC-P1 cells cocultivated with libraries of these virus-producing cells were independently infected with many retroviruses since hybridization of their genomic DNAs with probes yielded smears or multiple bands. To determine whether transduced FDC-P1 cells contained N2-IL3 proviral DNA, we digested their genomic DNAs with *Sac*I, thus releasing the intact viral genome, and hybridized the blots with IL3- or neomycin-specific probes. Figure 8b indicates that all these FI cell lines contained the 4.2-kb proviral IL3 sequence. DNA of cells from cultures of pN2-IL3 and pRN-BL3 at a ratio of 1:10,000 had a weak signal at the 4.2-kb band with the neomycin probe but not with the IL-3 probe. During repeated experiments with plasmids derived from bacteria of the same sublibrary, the 4.2-kb band probed with both neomycin and IL-3 was clearly present at that ratio. We also transduced another FD cell line, 32D,

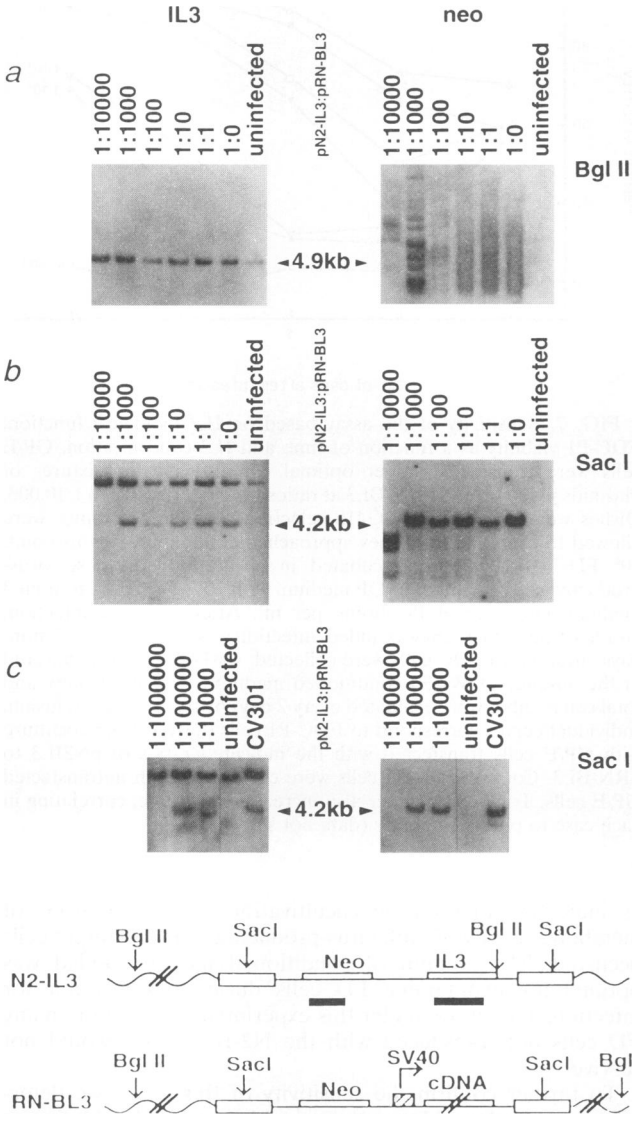

FIG. 8. Southern blot analysis. (a) Junction fragment analysis. DNA samples extracted from transduced FDC-P1 cell lines were digested with *Bgl*II. IL3-specific (left) and neomycin-specific (right) probes were used. The 4.9-kb bands correspond to the endogenous IL3 sequence. (b) Genomic DNA samples were digested with *Sac*I. Since the flanking retroviral long terminal repeats contain *Sac*I sites, this releases the integrated viral sequence. The DNA samples in this panel were derived from the corresponding cell cultures in Fig. 7. They were harvested after 6 (for 1:0, 1:1, and 1:10), 8 (for 1:100), 10 (1:1,000), 18 (1:10,000), and 22 days (for control). (c) Another FD cell line, 32D, was used for infection and was rendered FI by coculturing with GPE cells transduced with a mixture of N2-IL3 and a different sublibrary plasmid DNA of pRN-BL3. Genomic DNA samples were digested with *Sac*I. CV301, DNA from ψ2 packaging cells transfected with pN2-IL3, was used as a positive control; FDC, DNA from FDC-P1 cells, was used as negative control (uninfected). Other lanes contained DNAs from FI 32D cells cocultured with various ratios of pN2-IL3–pRN-BL3 plasmids. FI cells at ratios of 1:1,000,000, 1:100,000, and 1:10,000 were obtained after 70, 25, and 30 days in culture, respectively. In panels b and c, the 4.2-kb bands correspond to the integrated N2-IL3 viral sequence. Neo, neomycin; SV40, simian virus 40.

with GP/E cells transfected with plasmid DNA from a different pRN-BL3 sublibrary (pBL32), and Fig. 8c indicates that N2-IL3 proviral DNA was present at the 1:100,000 dilution. Of note was the fact that we obtained FI growth of 32D cells at the 1:1,000,000 dilution only after 70 days compared with less than 30 days for cultures with lower dilutions. Taken together, these data indicate that under near-optimal transfection conditions, the sensitivity of our assay can be as high as 1:100,000, i.e., a particular species of mRNA represented in a retroviral cDNA expression library at an abundance level as low as 0.001% can be isolated.

Conversion to FI after transduction with retroviral cDNAs. To further confirm the effectiveness of this methodology, we infected target FDC-P1 cells by cocultivating them with sublibraries of virus-producing cells. We observed that a subclone of FDC-P1 cells, FDE (Simon Jones, Genetic Institute), gave a low background of spontaneous FI growth; therefore, we used FDE cells as target cells for this series of experiments. Spontaneous mutation in FDE cells was estimated to be less than 1 in 10^7 cells (7a). Five types of retrovirus-producing cells were used: 7GN2, GP/E cells producing N2 virus; B2-22, B3-20, B6-35, and B6-45, GP/E cells producing four different sublibraries of pRN-cDNAs from BL3 cells. The efficiency of retroviral gene transfer into FDE cells ranged from 27%, the lowest, for one dish of B3-20 to 86%, the highest, for one dish of 7GN2 (Table 1).

To allow for the observation of reproducible independent infection events, duplicated dishes of each type of virus-producing cell were used for cocultivation and cells harvested from each dish were treated and cultured in methylcellulose separately. Table 1 indicates that dishes of both B2-22 and B3-20, but not B6-35, B6-45, or 7GN2, gave rise to FI colonies. It is important to point out that colonies from both dishes of B3-20 were qualitatively entirely different from those of B2-22. They were macroscopic in size, and 17 days after infection, they had proliferated to 2 to 6 million cells, whereas macroscopic colonies from dishes of other sublibraries cultured with growth factors had fewer than 30,000 cells. This type of macroscopic colony was not observed in any of the dishes from infection by eight other plates of retrovirus-producing cells. These data suggested that it was a cDNA in B3-20 whose product could uniquely stimulate transduced FDE cells to develop this type of colony.

FI colonies observed in cells transduced with retroviruses from B2-22 were typical colonies, appearing to be diffuse and with a center core. They did become macroscopic in size, but their rate of proliferation was far less rapid than that of those transduced with B3-20. The number of FI colonies per culture transduced by B2-22 cells was much more significant than that transduced by B3-20. These data suggested that the cDNA in B2-22, different from that in B3-20, was more abundant than that in B3-20 and that its expression also resulted in stimulating FI growth.

DISCUSSION

We have standardized the conditions for high-efficiency transfection of plasmids of a retroviral cDNA library stably into GP/E packaging cells. By assaying for FI growth of FD cells after retrovirus-mediated gene transfer, we were able to isolate IL-3 transduced cells when the vector carrying IL-3 cDNA was represented in the cDNA library at a frequency as low as 0.001%. This efficiency was based on transfecting vector DNA into two 100-mm-diameter tissue culture dishes so that an average of 4,000 independent clones of retroviral library transfectants were present (Fig. 5). Realistically, one can scale

TABLE 1. Infection of FDE cells with sublibraries of retrovirus-producing cells[a]

Plasmid sublibrary	Cell yield per dish (10^6)	No. of colonies/1,000 cells		No. of colonies/1.5 × 10^6 cells[b]		Efficiency of retroviral transduction (%)[c]
		+ GF, − G418	+ GF, + G418	− GF, − G418	− GF, + G418	
7GN2	5.4	620	528	0	0	85
	3.5	399	344	0.9 (1)	0	86
B2-22	4.1	559	270	13.7 (18)	6.9 (9)	48
	3.0	554	206	12.8 (12)	7.5 (7)	37
B3-20	8.2	650	221	2.3 (6)[d]	0	34
	4.8	656	174	0.7 (1)[d]	0	27
B6-35	6.2	643	198	0	0	31
	3.1	556	198	0	0	37
B6-45	5.5	668	268	0	0	40
	4.0	413	160	0	0	39

[a] FDE cells were cocultivated with virus-producing cells, and each row represents the results of a separate infection. Nonadherent cells were harvested and plated in methylcellulose cultures. For each harvest, 1,000 cells were plated into two dishes of cultures with (+) growth factor (GF) (WEHI-3 CM); remaining cells were divided equally and plated at a cell concentration of 5 × 10^5/ml in cultures without (−) GF and with (+) or without (−) 1 mg of G418 per ml. Total number of dishes prepared for cultures with no GF was a function of cell yield since all cells harvested were plated into methylcellulose cultures. For cultures with no GF, number of colonies per 1.5 × 10^6 cells was used because no colonies could be observed when 1.5 × 10^6 cells were plated into three dishes; all zero values for other points had more dishes since more cells were available for plating.

[b] Numbers in parentheses represent actual total numbers of colonies observed in dishes for each culture condition. 7GN2 is an N2 producer tested negative for helper virus by XC plaque assay (56).

[c] Calculated by dividing the numbers of colonies in cultures with growth factor (GF) and G418 by the numbers of colonies in cultures with GF and without G418 and then multiplying by 100.

[d] Macroscopic colonies qualitatively different from those derived from FDE cocultivated with other retroviral cultures.

up the screening effort easily by expanding the number of dishes, for example, to 20. Accordingly, the sensitivity of the method increases 10-fold; in other words, mRNA at 0.0001% can be isolated by this method.

Amplification also took place during cocultivation between target cells and virus-producing cells, which occurred for a period of 3 days. Depending on the experiment, there were between 10,000 and 100,000 different types of retroviruses produced in a dish containing an average of 4,000 independent transfectants, each of which had to produce more than one type of retrovirus (Fig. 7 and 8). As the exposure time of target cells to virus-producing cells increased, successful transduction by a retrovirus vector carrying a cDNA with growth-stimulating function also increased. As a result, this particular clone of FD cells transduced with the correct cDNA would have a distinct selective growth advantage over those transduced with genes having no growth-stimulating function. This type of clonal amplification should enhance the probability of detection.

Despite the low frequency of recombination for production of Moloney murine leukemia virus (40), a replication-competent virus may still be present and be a concern. However, the presence of such a helper virus may actually help to spread the desired construct into a larger number of target cells and lead to preferential expansion, thus providing another level of amplification. Genomic DNA from cells transduced with and stimulated by the product of the desired construct, either introduced initially or acquired through subsequent virus spread, should reveal a band of the same size upon *Sac*I digestion, consistent with the results in Fig. 8, whereas independent FI clones generated as a result of other mutational events would not. Also, if the helper virus is present in large quantities, prolonged cocultivation would not increase the frequency of infection due to viral interference. In our experiments, a prolonged time of infection resulted in increased efficiency of retroviral gene transfer (63). Therefore, a replication-competent helper virus could be present but only at very low levels, if at all; furthermore, its presence would only facilitate the screening process.

In the system we tested, one may identify genes capable of converting FD cells to FI growth. In this regard, it should facilitate our effort to identify new growth-stimulating genes from immortalized BL3 cells, which we recently established retain certain properties of hemopoietic stem cells (66). Retroviral transduction of FD cells with sublibraries of pRN cDNA from BL3 cells resulted in the development of different types of FI colonies (Table 1). The type of FI colonies observed in cultures of B3-20 were striking in that they were not observed in cells from eight other independent infection cultures and that the cells in these colonies proliferated at a rate significantly faster than, for example, those in FI colonies from infection cultures of B2-22 (Table 1). These data strongly suggested that the two cDNAs separately inserted into the retroviral vector were distinct from each other. Molecular isolation and characterization of the two cDNA are in progress. We were encouraged by this result because the results of RT-PCR and cell proliferation assay indicate that BL3 cells do not produce or respond to many cytokines (22), including IL-1 to IL-7, IL-9 to IL-11, stem cell factor, leukemia inhibitory factor, GM-CSF, granulocyte colony-stimulating factor, macrophage colony-stimulating factor, oncostatin-M, gamma interferon, and tumor necrosis factor alpha. However, they do produce an autocrine growth factor (22). The effects of the proteins of the isolated cDNAs on hemopoietic stem cells will be analyzed in a future study.

In our experimental protocol, plasmid DNAs of various sublibraries of pRN-BL3 were stably incorporated into the genome of GP/E transfectants because they were selected with G418. As such, cells from several dishes, each of which contained an average of 2 to 4 thousand independent colonies, were expanded, pooled, and frozen in a large number of vials as stocks for each retroviral sublibrary. When needed, cells of a sublibrary of interest were thawed out to infect target cells.

One such experiment was performed, and the results are presented in Table 1. In this way, we circumvented the need for extensive labor and yet provided consistent stocks of various sublibraries of retrovirus-producing cells.

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REFERENCES

1. **Aruffo, A., and B. Seed.** 1987. Molecular cloning of a CD28 cDNA by a high-efficiency COS cell expression system. Proc. Natl. Acad. Sci. USA **84:**8573–8577.
2. **Belmont, J. W., J. Henkel-Tigges, S. M. W. Chang, K. Wager-Smith, R. E. Kellems, J. E. Dick, M. C. Magli, R. A. Phillips, A. Bernstein, and C. T. Caskey.** 1986. Expression of human adenosine deaminase in murine haematopoietic progenitor cells following retroviral transfer. Nature (London) **322:**385–387.
3. **Blasi, E., B. J. Matheson, L. Varesio, J. L. Cleveland, A. Borchert, and U. R. Rapp.** 1985. Selective immortalization of murine macrophages from fresh bone marrow by a raf/myc recombinant murine retrovirus. Nature (London) **318:**667–670.
4. **Boswell, H. S., T. S. Nahreini, G. S. Burgess, A. Srivastava, T. G. Gabig, L. Inhorn, E. F. Srour, and M. A. Harrington.** 1990. A RAS oncogene imparts growth factor independence to myeloid cells that abnormally regulate protein kinase C: a nonautocrine transformation pathway. Exp. Hematol. **18:**452–460.
5. **Boutler, C. A., and E. F. Wagner.** 1988. The effects of v-src expression on the differentiation of embryonal carcinoma cells. Oncogene **2:**207–214.
6. **Browder, T. M., J. S. Abrams, P. M. C. Wong, and A. W. Nienhuis.** 1989. Mechanism of autocrine stimulation in hematopoietic cells producing interleukin-3 after retrovirus-mediated gene transfer. Mol. Cell. Biol. **9:**204–213.
7. **Chang, J. M., D. Metcalf, R. A. Lang, T. J. Gonda, and G. R. Johnson.** 1989. Nonnioplastic hematopoietic myeloproliferative syndrome induced by dysregulated multi-CSF (IL-3) expression. Blood **73:**1487–1497.

7a. **Chung, S.-W.** Unpublished data.

8. **Chung, S. W., P. M. C. Wong, H. Durkin, Y. Wu, and J. Petersen.** 1991. Leukemia initiated by hemopoietic stem cells expressing the v-abl oncogene. Proc. Natl. Acad. Sci. USA **88:**1585–1589.
9. **Chung, S. W., P. M. C. Wong, G. Shen-Ong, S. Ruscetti, T. Ishizaka, and C. J. Eaves.** 1986. Production of granulocyte-macrophage colony-stimulating factor by abelson virus-induced tumorigenic mast cell lines. Blood **68:**1074–1081.
10. **Cone, R. D., E. B. Reilly, H. N. Eisen, and R. C. Mulligan.** 1987. Tissue-specific expression of functionally rearranged λ1 Ig gene through a retrovirus vector. Science **236:**954–957.
11. **Cook, W. D., D. Metcalf, N. A. Nicola, A. W. Burgess, and F. Walker.** 1985. Malignant transformation of a growth factor-dependent myeloid cell line by Abelson virus without evidence of an autocrine mechanism. Cell **41:**677–683.
12. **Dean, M., J. L. Cleveland, U. R. Rapp, and J. N. Ihle.** 1987. Role of myc in the abrogation of IL3 dependence of myeloid FDC-P1 cells. Oncogene Res. **1:**279–287.
13. **Dexter, T. M., J. Garland, D. Scott, E. Scolnick, and D. Metcalf.** 1980. Growth of factor-dependent hemopoietic precursor cell lines. J. Exp. Med. **152:**1036–1047.
14. **Dick, J. E., M. C. Magli, D. Huszar, R. A. Phillips, and A. Bernstein.** 1985. Introduction of a selectable gene into primitive stem cells capable of long-term reconstitution of the hemopoietic system of W/W^v mice. Cell **42:**71–79.
15. **Dzierzak, F. A., T. Papayannopoulou, and R. C. Mulligan.** 1988. Lineage-specific expression of a human β-globin gene in murine bone marrow transplant recipients reconstituted with retrovirus-transduced stem cells. Nature (London) **331:**35–41.
16. **Eglitis, M. A., P. Kantoff, E. Gilboa, and W. F. Anderson.** 1985. Gene expression in mice after high efficiency retroviral gene transfer. Science **230:**1395–1398.
17. **Friedman, R. L.** 1985. Expression of human adenosine deaminase using a transmissable murine retrovirus vector system. Proc. Natl. Acad. Sci. USA **82:**703–707.
18. **Funkunaga, R., E. Ishizaka-Ikeda, Y. Seto, and S. Nagata.** 1990. Expression cloning of a receptor for murine granulocyte colony-stimulating factor. Cell **61:**341–350.
19. **Gearing, D. P., J. A. King, N. M. Gough, and N. A. Nicola.** 1989. Expression cloning of a receptor for human granulocyte-macrophage colony-stimulating factor. EMBO J. **8:**3667–3676.
20. **Gonda, T. J., D. K. Sheiness, and J. M. Bishop.** 1989. Murine myeloid cell lines derived by in vitro infection with recombinant c-myb retroviruses express myb from rearranged vector proviruses. EMBO J. **8:**1767–1775.
21. **Gruber, H. E., K. D. Finley, R. M. Hershberg, S. S. Katzman, P. K. Laikind, J. E. Seegmiller, T. Friedmann, J. K. Yee, and D. J. Jolly.** 1985. Retroviral vector-mediated gene transfer into human hematopoietic progenitor cells. Science **230:**1057–1061.
22. **Han, X. D., and P. M. C. Wong.** Unpublished data.
23. **Hanahan, D.** Studies on transformation of Escherichia coli with plasmids. J. Mol. Biol. **166:**557–580.
24. **Hariharan, I. K., J. M. Adams, and S. Cory.** 1988. bcr-abl oncogene renders myeloid cell line factor independent: potential autocrine mechanism in chronic myeloid leukemia. Oncogene Res. **3:**387–399.
25. **Heard, J. M., M. F. Roussel, C. W. Rettenmier, and C. J. Sherr.** 1987. Multilineage hematopoietic disorders induced by transplantation of bone marrow cells expressing the v-fms oncogene. Cell **51:**663–673.
26. **Johnson, G. R., T. J. Gonda, D. Metcalf, I. K. Hariharan, and S. Cory.** 1989. A lethal myeloproliferative syndrome in mice transplanted with bone marrow cells infected with a retrovirus expressing granulocyte-macrophage colony stimulating factor. EMBO J. **8:**441–448.
27. **Kato, J., M. F. Roussel, R. A. Ashmun, and C. J. Sherr.** 1989. Transduction of human colony-stimulating factor-1 (CSF-1) receptor into interleukin-3-dependent mouse myeloid cells induces both CSF-1-dependent and factor-independent growth. Mol. Cell. Biol. **9:**4069–4073.
28. **Keller, G., C. Paige, E. Gilboa, and E. Wagner.** 1985. Expression of a foreign gene in myeloid and lymphoid cells derived from multipotent haematopoietic precursors. Nature (London) **318:** 149–154.
29. **Keller, J. R., S. K. Ruscetti, and F. W. Ruscetti.** 1990. Introduction of v-abl oncogene induces monocytic differentiation of an IL-3-dependent myeloid progenitor cell line. Oncogene **5:**549–555.
30. **Laker, C., C. Stocking, U. Bergholz, N. Hess, J. F. De Lamarter, and W. Ostertag.** 1987. Autocrine stimulation after transfer of the granulocyte/macrophage colony-stimulating factor gene and autonomous growth are distinct but interdependent steps in the oncogenic pathway. Proc. Natl. Acad. Sci. USA **84:**8458–8462.
31. **Lang, R. A., D. Metcalf, N. M. Gough, A. R. Dunn, and T. I. Gonda.** 1985. Expression of a hemopoietic growth factor cDNA in a factor-dependent cell line results in autonomous growth and tumorigenicity. Cell **43:**531–542.
32. **Langdon, W. Y., J. W. Hartley, S. P. Klinken, S. K. Ruscetti, and H. C. Morse III.** 1989. v-cbl, an oncogene from a dual-recombinant murine. Proc. Natl. Acad. Sci. USA **86:**1168–1172.
33. **Ledley, F. D., G. J. Darlington, T. Hahn, and S. L. C. Woo.** 1987. Retroviral gene transfer into primary hepatocytes: implications for genetic therapy of liver-specific functions. Proc. Natl. Acad. Sci. USA **84:**5335–5339.
34. **Ledley, F. D., H. E. Grenett, M. McGinnis-Shelnutt, and S. L. C. Woo.** 1986. Retroviral-mediated gene transfer of human phenylalanine hydroxylase into NIH 3T3 and hepatoma cells. Proc. Natl. Acad. Sci. USA **83:**409–413.
35. **Lee, F., T. Yokota, T. Otsuka, L. Gemmel, N. Larson, J. Luh, K. I. Arai, and D. Rennick.** 1985. Isolation of cDNA for a human granulocyte-macrophage colony-stimulating factor by functional expression in mammalian cells. Proc. Natl. Acad. Sci. USA **82:**4360–4364.

36. **Lee, F., T. Yokota, T. Otsuka, P. Meyerson, D. Villaret, R. Coffman, T. Mosmann, D. Rennick, N. Roehm, C. Smith, A. Zlotnik, and K. I. Arai.** 1986. Isolation and characterization of a mouse interleukin cDNA clone that expresses B-cell stimulatory factor 1 activities and T-cell- and mast-cell-stimulating activities. Proc. Natl. Acad. Sci. USA **83:**2061–2065.
37. **Le Gros, G., S. Gillis, and J. Watson.** 1985. Induction of IL 2 responsiveness in a murine IL 3-dependent cell line. J. Immunol. **135:**4009–4014.
38. **Li, M., and O. Bernard.** 1992. FDC-P1 myeloid cells engineered to express fibroblast growth factor receptor 1 proliferate and differentiate in the presence of fibroblast growth factor and heparin. Proc. Natl. Acad. Sci. USA **89:**3315–3319.
39. **Lim, B., D. A. Williams, and S. H. Orkin.** 1987. Retrovirus-mediated gene transfer of human adenosine deaminase: expression of functional enzyme in murine hematopoietic stem cells in vivo. Mol. Cell. Biol. **7:**3459–3465.
40. **Markowitz, D., S. Goff, and A. Bank.** 1988. A safe packaging line for gene transfer: separating viral genes on two different plasmids. J. Virol. **62:**1120–1124.
41. **Matsui, T., J. H. Pierce, T. P. Fleming, J. S. Greenberger, W. J. LaRochelle, M. Ruggiero, and S. A. Aaronson.** 1989. Independent expression of human alpha or beta platelet-derived growth factor receptor cDNAs in a naive hematopoietic cell leads to functional coupling with mitogenic and chemotactic signaling pathways. Proc. Natl. Acad. Sci. USA **86:**8314–8318.
42. **McCubrey, J. A., S. L. Steelman, M. W. Mayo, P. A. Algate, R. A. Dellow, and M. Kaleko.** 1991. Growth-promoting effects of insulin-like growth factor-1 (IGF-1) on hematopoietic cells: overexpression of introduced IGF-1 receptor abrogates interleukin-3 dependency of murine factor-dependent cells by a ligand-dependent mechanism. Blood **78:**921–929.
43. **Meckling-Gill, K. A., S. Yee, J. W. Schrader, and T. Pawson.** 1992. A retrovirus encoding the v-fps protein-tyrosine kinase induces factor-dependent growth and tumorigenicity in FDC-P1 cells. Biochim. Biophys. Acta **1137:**65–72.
44. **Migliaccio, A. R., G. Migliaccio, A. D'Andrea, M. Baiocchi, S. Crotta, S. Nicolis, S. Ottolenghi, and J. W. Adamson.** 1991. Response to erythropoietin in erythroid subclones of the factor-dependent cell line 32D is determined by translocation of the erythropoietin receptor to the cell surface. Proc. Natl. Acad. Sci. USA **88:**11086–11090.
45. **Noma, Y., P. Sideras, T. Naito, S. Bergstedt-Lindquist, C. Azuma, E. Severinson, T. Tanabe, T. Kinashi, F. Matsuda, Y. Yaoita, and T. Honjo.** 1986. Cloning of cDNA encoding the murine IgG1 induction factor by a novel strategy using SP6 promoter. Nature (London) **319:**640–646.
46. **Okayama, H., and P. Berg.** 1982. High-efficiency cloning of full-length cDNA. Mol. Cell. Biol. **2:**161–170.
47. **Oliff, A., O. Agranovsky, M. D. McKinney, V. V. V. S. Murty, and R. Bauchwitz.** 1985. Friend murine leukemia virus-immortalized myeloid cells are converted into tumorigenic cell lines by Abelson leukemia virus. Proc. Natl. Acad. Sci. USA **82:**3306–3310.
48. **Otani, H., J. P. Siegel, M. Erdos, J. R. Gnarra, M. B. Toledano, M. Sharon, H. Mostowski, M. B. Feinberg, J. H. Pierce, and W. J. Leonard.** 1992. Interleukin (IL)-2 and IL-3 induce distinct but overlapping responses in murine IL-3-dependent 32D cells transduced with human IL-2 receptor β chain: involvement of tyrosine kinase(s) other than $p56^{lck}$. Proc. Natl. Acad. Sci. USA **89:**2789.
49. **Paul, W. E.** 1989. Pleiotropy and redundancy: T cell-derived lymphokines in the immune response. Cell **57:**521–524.
50. **Pierce, J. H., P. P. DiFiore, S. A. Aaronson, M. Potter, J. Pumphrey, A. Scott, and J. N. Ihle.** 1985. Neoplastic transformation of mast cells colonies. Cell **41:**685–693.
51. **Pierce, J. H., M. Ruggiero, T. P. Fleming, P. P. DiFiore, J. S. Greenberger, L. Varticovski, J. Schlessinger, J. Rovera, and S. A. Aaronson.** 1988. Signal transduction through the EGF receptor transfected in IL-3-dependent hematopoietic cells. Science **239:** 628–631.
52. **Quelle, D. E., and D. M. Wojchowski.** 1991. Localized cytosolic domains of the erythropoietin receptor regulate growth signaling and down-modulate responsiveness to granulocyte-macrophage colony-stimulating factor. Proc. Natl. Acad. Sci. USA **88:**4801–4805.
53. **Rapp, U. R., J. L. Cleveland, K. Brightman, A. Scott, and J. N. Ihle.** 1985. Abrogation of IL-3 and IL-2 dependence by recombinant murine retroviruses expressing v-myc oncogenes. Nature (London) **317:**434–438.
54. **Rein, A., J. Keller, A. M. Schultz, K. L. Holmes, R. Medicus, and J. N. Ihle.** 1985. Infection of immune mast cells by Harvey sarcoma virus: immortalization without loss of requirement for interleukin-3. Mol. Cell. Biol. **5:**2257–2264.
55. **Rohrschneider, I. R., and D. Metcalf.** 1989. Induction of macrophage colony-stimulating factor-dependent growth and differentiation after introduction of the murine c-*fms* gene into FDC-P1 cells. Mol. Cell. Biol. **9:**5081–5092.
56. **Rowe, W. P., W. E. Pugh, and J. W. Hartley.** 1970. Plaque assay techniques for murine leukemia viruses. Virology **42:**1136–1139.
57. **Ruscetti, S., R. Aurigemma, C.-C. Yuan, S. Sawyer, and D. G. Blair.** 1992. Induction of erythropoietin responsiveness in murine hematopoietic cells by the *gag-myb-ets*-containing ME26 virus. J. Virol. **66:**20–26.
58. **Sorge, J., W. Kuhl, C. West, and E. Beutler.** 1987. Complete correction of the enzymatic defect of type I Gaucher disease fibroblasts by retroviral-mediated gene transfer. Proc. Natl. Acad. Sci. USA **84:**906–909.
59. **Sorge, J., D. Wright, V. D. Erdman, and A. E. Cutting.** 1984. Amphotropic retrovirus vector system for human cell gene transfer. Mol. Cell. Biol. **4:**1730–1737.
60. **Takaki, S., A. Tominaga, Y. Hitosh, S. Mita, E. Sonoda, N. Yamaguchi, and K. Takatsu.** 1990. Molecular cloning and expression of the murine interleukin-5 receptor. EMBO J. **9:**4367–4374.
61. **Valtieri, M., D. J. Tweardy, D. Caracciolo, K. Johnson, F. Mavilio, S. Altman, D. Santoli, and G. Rovera.** 1987. Cytokine-dependent granulocytic differentiation: regulation of proliferative and differentiative responses in a murine progenitor cell line. J. Immunol. **138:**3829–3835.
62. **Wang, B., W. S. Kennan, J. Yasukawa-Barnes, M. J. Lindstrom, and M. N. Gould.** 1991. Overcoming the activity of mammary carcinoma suppressor gene in Copenhagen rats by v-H-ras oncogene transfer into mammary epithelial cells in situ. Cancer Res. **51:**2642–2648.
63. **Wong, B. Y., and P. M. C. Wong.** Unpublished data.
64. **Wong, P. M. C., S. W. Chung, C. E. Dunbar, D. M. Bodine, S. K. Ruscetti, and A. W. Nienhuis.** 1989. Retrovirus-mediated transfer and expression of the interleukin-3 gene in mouse hematopoietic cells result in a myeloproliferative disorder. Mol. Cell. Biol. **9:**798–808.
65. **Wong, P. M. C., S. W. Chung, and A. W. Nienhuis.** 1987. Retroviral transfer and expression of the interleukin-3 gene in hemopoietic cells. Genes Dev. **1:**358–365.
66. **Wong, P. M. C., X. D. Han, F. Ruscetti, and S. W. Chung.** Immortalized hemopoietic cells with stem cell properties. Submitted for publication.
67. **Wongsasant, B., S. Matsuda, and T. Yamamoto.** 1991. Active c-erbB-2 induces short-term growth of FDC-P2 cells after IL-3 depletion. Biochem. Biophys. Res. Commun. **181:**981–988.
68. **Yokota, T., N. Arai, F. Lee, D. Rennick, T. Mosmann, and K. I. Arai.** 1985. Use of a cDNA expression vector for isolation of mouse interleukin 2 cDNA clones: expression of T-cell growth-factor activity after transfection of monkey cells. Proc. Natl. Acad. Sci. USA **82:**68–72.
69. **Yokota, T., F. Lee, D. Rennick, C. Hall, N. Arai, T. Mosmann, G. Nabel, H. Cantor, and K. Arai.** 1984. Isolation and characterization of a mouse cDNA clone that expresses mast-cell growth-factor activity in monkey cells. Proc. Natl. Acad. Sci. USA **81:**1070–1074.