Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 2411–2415, April 1987
Genetics

Retroviral-mediated transfer of genomic globin genes leads to regulated production of RNA and protein

(retroviral vectors/mouse erythroleukemia cells/gene regulation/antisense RNA/gene therapy)

STEFAN KARLSSON*†, THALIA PAPAYANNOPOULOU‡, STEFAN G. SCHWEIGER*, GEORGE STAMATOYANNOPOULOS‡, AND ARTHUR W. NIENHUIS*

*Clinical Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892; and ‡Department of Medicine, University of Washington School of Medicine, Seattle, WA 98195

Communicated by Roscoe O. Brady, December 22, 1986 (received for review September 25, 1986)

ABSTRACT **A high-titer amphotropic retroviral vector containing the neomycin resistance gene and a hybrid γ-β genomic human globin gene has been constructed. Mouse erythroleukemia cells infected with this virus were found to contain the full transcriptional unit of the transferred human globin gene by Southern blot analysis. These cells contain normally initiated, spliced, and terminated human globin mRNA. The human globin mRNA level increased 5- to 10-fold upon induction of the mouse erythroleukemia cells. Human globin chains were produced but only in a fraction of the cells as detected by immunofluorescent staining. A similar retrovirus containing a human β-globin gene was used to transduce mouse erythroleukemia cells resulting in much higher levels of human globin synthesis than detected in mouse erythroleukemia cells transduced with the γ-β globin virus.**

Retroviruses are animal viruses with an RNA genome that can infect a wide variety of host cells. After entering cells, the RNA genome is reverse transcribed into DNA, and the double-stranded DNA intermediate integrates with high efficiency into cellular DNA creating the provirus (1). Integration occurs at specific sites in the viral genome but the insertion sites in the cellular genome are random or semirandom (2). A large proportion of the retroviral genome can be replaced by inserted genes without compromising their ability to become encapsidated into virions. Furthermore, helper cell lines (3, 4) can package recombinant viruses without contamination by a helper virus. The wide host range, efficient integration mechanisms, and packaging of helper-free recombinant viruses make retroviruses prime candidates to deliver genes efficiently into cultured cells and whole organisms (5, 6).

Retroviral vectors have been used successfully to deliver genes into hematopoietic stem cells of mice (7–10), into hematopoietic progenitor cells of man (11, 12), and into fertilized mouse eggs to achieve germ-line gene transfer (13–15). To achieve efficient gene replacement therapy using retroviral vectors (16), effective delivery of the gene is a necessary but not a sufficient condition. In addition the gene has to function efficiently and appropriately. A large body of information exists about the effective delivery of genes using retroviral vectors (5, 6) but less is known about appropriate regulation of the transferred genes when they are controlled by tissue-specific promoters. A virus containing a rat growth hormone minigene has been shown to produce inducible rat growth hormone mRNA production (17). A genomic globin gene has been inserted into a retrovirus, transferred into mouse erythroleukemia (MEL) cells, and shown to result in regulated production of human globin mRNA, but at very low levels (18). We show here that a different retrovirus, based on the N2 vector (9, 10), containing a genomic human globin gene can, after transfer into MEL cells, express high levels of mRNA in a regulated fashion and in addition lead to production of the human globin polypeptide chain in induced MEL cells.

METHODS

Cells and Viruses. All cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% (vol/vol) fetal bovine serum (Hyclone) except ψ2 cells (3), obtained from R. C. Mulligan, that were grown in 10% (vol/vol) calf serum (Colorado Serum, Denver, CO). Cell lines for titering viruses (thymidine kinase-negative 3T3 cells, CCC^- cells, and NRK cells) and amphotropic packaging cell lines PA 12 and PA 317 were obtained from A. D. Miller (4, 19). Diploid and tetraploid MEL cells (obtained from A. Deisseroth) were used (20). Viruses containing the neomycin-resistance (neo^R) gene were titered on thymidine kinase-negative 3T3 cells (4) using 1 g (dry weight) of G418 per liter (GIBCO). Amphotropic helper virus was detected using the S^+L^- assay (4).

Vector Construction. The plasmid XM5 containing the N2 provirus was provided by E. Gilboa (9, 10). A 2.5-kilobase (kb) hybrid human γ-β globin gene was made by ligating a fragment of the ^{G}γ-globin gene [from the *Stu* I site 386 base pairs (bp) upstream from the cap site to the *Bam*HI site at the end of exon 2] to a 3′ fragment of the human β-globin gene (from the intragenic *Bam*HI site to the *Pst* I site 689 bp downstream from the β-globin gene-coding sequence). This hybrid γ-β gene was inserted into the *Xba* I site of pUC12 and excised with *Sma* I and *Hin*cII to create a γ-β globin gene with blunt ends that was inserted into the blunted *Xho* I site of the N2 provirus to create the neo^R-globin (NG) virus (Fig. 1). A similar retroviral plasmid containing a 3.4-kb human β-globin gene (*Apa* I site at position −1250 to the *Pst* I site 689 bp downstream from the β-globin gene-coding sequence) was also constructed (β-globin virus). Plasmids having the globin gene inserted in an opposite orientation to the direction of viral genomic mRNA transcription were selected (Fig. 1) to avoid rearrangements of the inserted gene or splicing out of the globin gene introns during the RNA phase of the viral infection cycle (21). Plasmid DNA was introduced into ψ2 ecotropic helper cells by calcium phosphate-mediated DNA transfer, and 2 days later the culture supernatant was used to infect PA 12 amphotropic packaging cells (NG virus) or PA 317 packaging cells (β-globin virus) (4, 19). MEL cells were infected with the amphotropic NG virus or the β-globin virus

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: MEL, mouse erythroleukemia cells; neo^R gene, neomycin-resistance gene; HMBA, hexamethylene bisacetamide; NG, neo^R-globin.

†Present address: Developmental and Metabolic Neurology Branch, National Institute of Neurological Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892.

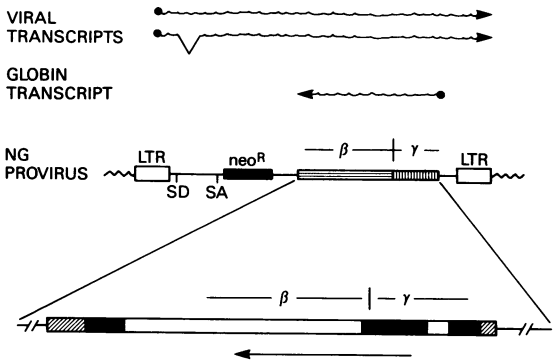

FIG. 1. A diagram of the NG virus. The expanded region of the 2.5-kb γ-β globin gene shows the exons (dark boxes), introns (empty boxes), and the 5'- and 3'-noncoding regions (hatched boxes). The γ-β globin gene is inserted in an opposite orientation to the direction of viral transcription (arrow). The main transcripts of the NG virus are also shown. Another virus analogous to the NG virus but containing a 3.4-kb human β-globin gene instead of the 2.5-kb γ-β gene was also constructed and is referred to as the β-globin virus. SD, splice donor; SA, splice acceptor.

by coculture for 4–18 hr in the presence of polybrene at 5 μg/ml followed by selection in G418 [1 g/liter (dry weight)]. Individual clones were isolated by limiting dilution.

DNA and RNA Analysis. Southern blot and RNA gel blot analyses were by described methods (20, 22). S1 nuclease mapping of total cellular RNA (23) was performed using single-stranded ^{32}P-labeled DNA probes (24). The probe fragments were inserted into M13 or the pTZ18R and pTZ19R plasmids (Pharmacia), and their single-stranded forms were used to synthesize the labeled probe using a primer extended by the Klenow fragment of DNA polymerase I (New England Biolabs).

Detection of Globin Chains. Human globin chains were detected by immunofluorescent staining. Cytocentrifuge prepared smears of MEL cells were fixed in methanol and labeled with monoclonal anti-human γ-chain antibody or anti-human β-chain antibodies (25) followed by fluorescein-conjugated goat anti-mouse IgG [F(ab')$_2$], (Tago, Burlingame, CA). Isoelectric focusing was used to compare the relative synthesis of mouse and human globin chains (26).

RESULTS

The NG Vector. The NG vector contains two promoters, the viral long terminal repeat and the human γ-globin promoter, in opposing transcriptional orientations (Fig. 1). The neo^R gene is driven by the long terminal repeat promoter, and the γ-β globin gene is driven by the γ-globin promoter. A cell clone called G5 that produces NG virus to high titer (2×10^6 colony-forming units/ml when titered on thymidine kinase negative-3T3 cells selected in G418) was used in all experiments presented here. Two of ≈10 virus-producing clones had the intact γ-β globin gene and a titer $>10^5$ colony-forming units/ml. The helper genome in PA 12 cells has been shown to recombine with N2-based vectors (4); the G5 clone produced helper virus with a titer of 10^4 focus-forming units/ml using the S^+L^- assay (4). The β-globin virus was packaged in PA 317 cells (19) and is helper free. Three of 12 clones contained the unrearranged β-globin gene (titer range $2–20 \times 10^3$ colony-forming units/ml), and two of them, β-2 and β-12, were used in the experiments described here.

High Levels of Normally Initiated, Spliced, and Terminated γ-β mRNA Are Produced in NG-Transduced MEL Cells. MEL cells are murine erythroid cells transformed by the Friend retrovirus that are arrested at a relatively late stage in erythroid differentiation (27). Chemical inducers can result in further differentiation of these cells leading to efficient expression of endogenous adult α- and β-globin genes and

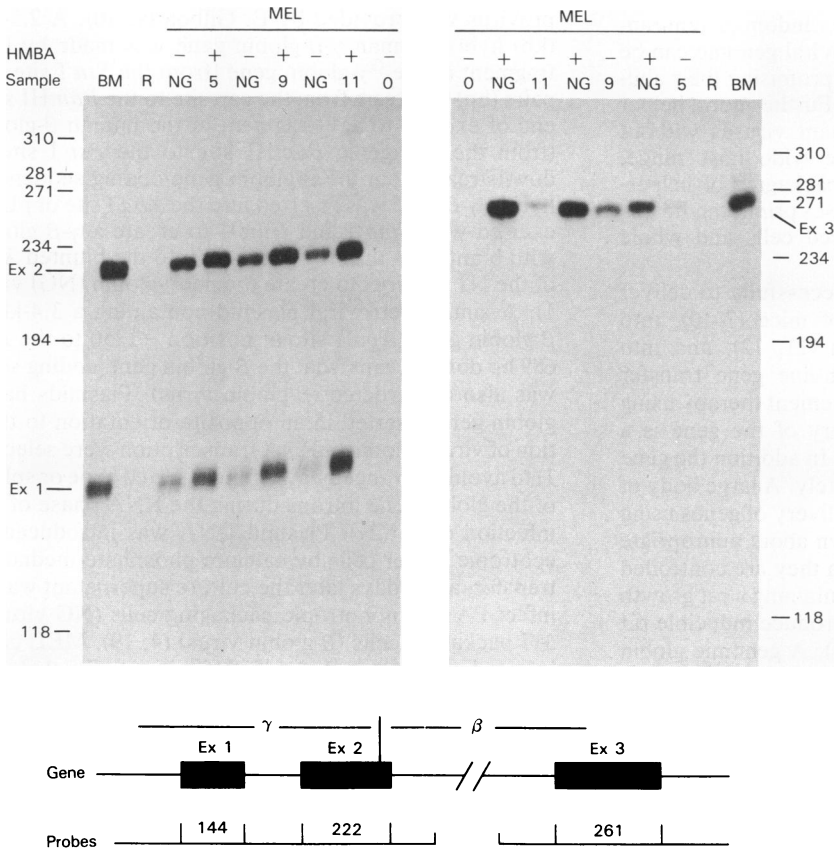

FIG. 2. S1 nuclease analysis of RNA samples from MEL clones transduced with the NG virus. Three clones NG5, NG9, and NG11 are analyzed in an induced (+) or an uninduced (−) state. R, tRNA sample; BM, human bone marrow RNA. Twenty micrograms of RNA was analyzed except for BM when 2 μg was used. Size markers in nucleotides are shown. The gene analyzed and the probes used are as follows: Analysis using the exon 1/exon 2 probe (650 bp) (*Upper Left*), which should result in protected fragments of 144 (Ex1) and 222 (Ex2) nucleotides (*Lower Left*). (*Right*) Analysis using the exon 3 probe (620 bp) resulting in a 261-nucleotide protected fragment.

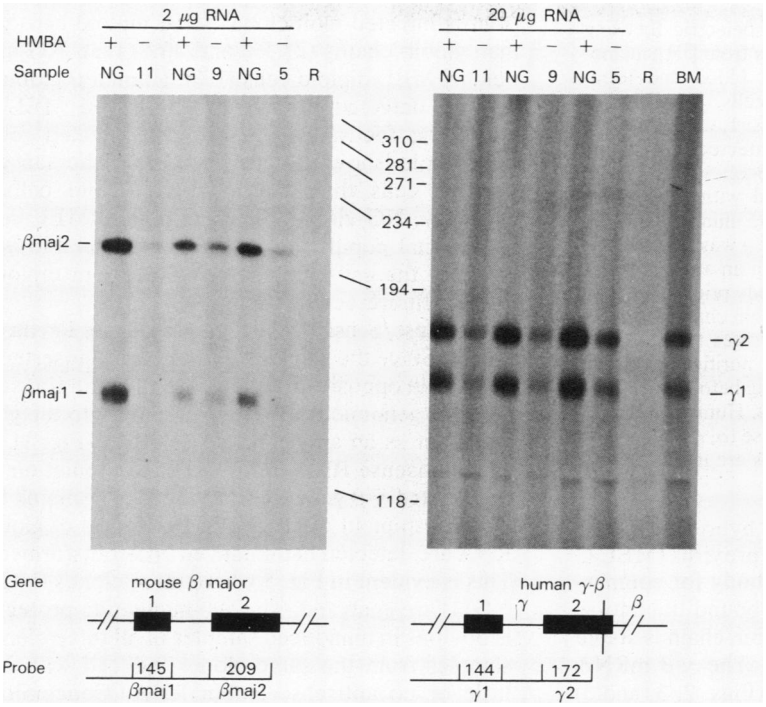

FIG. 3. This S1 nuclease assay shows a quantitative comparison of mouse β^{major} and human γ-β mRNA in three clones of NG-transformed MEL cells. The samples are the same as in Fig. 2. The probes used are indicated below. (*Left*) RNA (2 μg) is analyzed with the mouse probe (1.7 kb). (*Right*) The same RNA sample (20 μg) is analyzed with a human γ-globin probe (569 bp). Two different concentrations of RNA were used to ensure probe excess. Exposure times for both autoradiographs (*Left* and *Right*) are the same. Both probes were cloned into M13, labeled, and isolated as described (24).

also adult human globin genes introduced into these cells by cell fusion or DNA transfection (28). Cloned human β-globin and γ-β hybrid globin genes have been transferred into these cells and found to be expressed in a regulated fashion (29, 30).

NG virus-infected diploid MEL cells were selected in G418 and found to contain the full transcriptional unit of the γ-β globin gene integrated into the cellular chromosomes by Southern blot analysis (data not shown). S1 nuclease analysis of RNA from these nonclonal populations of MEL cells showed correctly initiated γ-β globin mRNA that can be increased 5- to 10-fold upon hexamethylene bisacetamide (HMBA) induction (data not shown). These results are consistent with studies reporting induction of γ-β globin genes transferred into MEL cells by DNA transfection (29, 30).

To determine whether there was clonal variability in the extent and nature of γ-β mRNA expression, individual clones were expanded in tissue culture, and RNA isolated from induced and uninduced cells was analyzed by S1 nuclease mapping (Fig. 2). Probes were made from the sequence of the hybrid gene itself to determine whether exon 1, 2, and 3 of the hybrid gene were appropriately protected in an S1 nuclease assay. As shown in Fig. 2 mRNA from all clones protected DNA fragments of sizes corresponding to exon 1 (144 bp), exon 2 (222 bp), and exon 3 (261 bp). There did not seem to be a major difference in γ-β mRNA levels among the three clones analyzed, all of which contain levels similar to the multiclonal population mentioned above.

We compared the human γ-β RNA level to that of the mouse β^{major} endogenous genes in the NG-transduced MEL cells by assaying different concentrations of RNA with probes for mouse and human globin transcripts. Fig. 3 shows an S1 nuclease mapping experiment analyzing RNA from the three MEL clones using both mouse β^{major} and human γ-β globin probes. It is clear that 2 μg of RNA gives a similar signal with the mouse probe as 20 μg gives with the human probe. In experiments using probes of similar specific activity with minimal probe reannealing and assaying more than one concentration of RNA to ensure probe excess, the γ-β mRNA level in induced MEL cells gave values of 7–40% of the mouse β^{major} mRNA by scanning densitometry.

Expression of Human Globin Chains in Induced MEL Cells. To determine whether the human γ-β mRNA leads to production of γ-β polypeptide chains, we used a monoclonal antibody against an epitope that included residue 45 in the human γ-globin chain. By immunofluorescence we could detect γ-β chains in induced but not in the uninduced NG-transformed MEL cells (Table 1). The proportion of cells that are stained with the antibody is low in the multiclonal population (about 1%) and quite variable from one clone to another. Two clones, NG5 and NG9, show 2–5% induction on day 3, which becomes 12–18% on days 5–8. Clone NG11 has <1% of the cells positive for human globin chains despite high levels of γ-β mRNA (Figs. 3 and 4). The viability of the cells remained high throughout the induction (Table 1).

A mixed population of tetraploid MEL cells (not clonal)

Table 1. Detection of the human γ-β globin chain in the induced MEL cells using an anti-γ-globin monoclonal antibody and immunofluorescent staining

		Globin synthesis, % positive cells						
Cell	Assay	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
MEL V6	Benzidine	92	—	—	—	—	—	—
	Antibody	0	0	0	0	0	0	0
MEL NG5	Benzidine	92	—	—	—	—	—	—
	Antibody	<1	4.5	11	18	13	—	7
MEL NG9	Benzidine	96	—	—	—	—	—	—
	Antibody	<1	2	11	12	12	—	18
MEL NG11	Benzidine	85	95	91	98	100	100	—
	Antibody	0	0	<1	<1	<1	—	—
MEL C	Benzidine	10.5	32	72	95	100	100	—
	Antibody	<1	7.5	40	60	60	60	60

MEL V6 cells represent a population infected with the N2 retrovirus that does not contain a globin gene. NG5, NG9, and NG11 are three different MEL clones transformed with the NG virus. MEL C is a mixed population of cells that was transfected with a human γ-β globin gene by the calcium phosphate technique (30). The benzidine staining is included to compare expression of the endogenous mouse hemoglobin with that of the transferred human γ-β gene. The viability of the cells remained 90–100% (closer to 100%) throughout the induction. The transfected MEL C cells are tetraploid, the other MEL cells are diploid. Immunofluorescence was as described (25, 26).

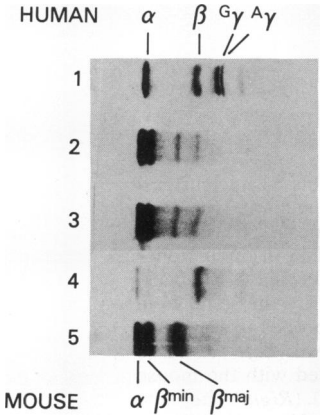

FIG. 4. Isoelectric focusing of cell lysates from [^{3}H]leucine-labeled cord blood and tetraploid MEL cells. The samples are as follows: 1, cord blood; 2, MEL cells infected with the β-virus clone β-12; 3 and 4, MEL cells infected with the β-virus clone β-2; 5, uninfected MEL cells. Sample 4 was immunoprecipitated with an anti-human β-globin antibody prior to analysis (25, 26). Globin chains from the other cell lysates (samples 1–3 and 5) were purified by haploglobin binding before isoelectric focusing (31). Human (α, β, ^{G}γ, ^{A}γ) and mouse (α, β^{minor}, β^{major}) globin chains are indicated.

transfected (calcium phosphate) with a γ-β hybrid gene (30), which is not cloned into a retroviral vector provirus (MEL C in Table 1), was also stained with the antibody for comparison. Forty to sixty percent of these cells bound the anti-γ-globin antibody, indicating that the γ-β globin chain is stable enough to be detected in the MEL cells. The γ-β mRNA levels in the NG-transduced MEL clones (Figs. 2, 3, and 5) are comparable to the γ-β mRNA levels in the transfected MEL cells (30).

The low levels of γ-β hybrid chains in the NG-infected diploid MEL cells could be, at least in part, due to lower stability of γ-β than β chains. β-globin viruses (β-2 and β-12) were used to infect MEL cells, and human β-globin chain production was analyzed in the transduced cells by immunofluorescent staining (Table 2). A higher proportion of β-virus-infected multiclonal MEL diploid cells contains human globin chains (27.5% and 46%) than NG-infected multiclonal MEL diploid cells (1%). Human β-globin chains can also be detected in β-virus transduced MEL cells using isoelectric focusing (Fig. 4). The infected tetraploid cell populations show a higher number of human globin chain positive cells than the infected diploid cells (Table 2). Similarly, NG virus-infected tetraploid MEL cells result in multiclonal populations that have detectable γ-β chains in 2–3% of the cells (compared with 1% in diploid MEL) by immunofluorescent staining.

Antisense/Sense RNA: Changes During Erythroid Differentiation. Since the genomic globin gene is inserted into the N2 virus in an opposite orientation to the viral transcription unit, the viral genomic RNA complementary to the globin mRNA could act as an antisense globin RNA (Fig. 1). There is no such antisense RNA in the MEL C population containing a transfected γ-β gene that produced γ-β chains in up to 60% of the tetraploid MEL cells. High levels of globin antisense RNA are detected in uninduced NG-transformed MEL cells. This is evident in Fig. 5 where three clones (NG5, NG9, and NG11) are analyzed. The 500-nucleotide protected fragment is obvious in uninduced samples of all three clones, but RNA samples from the same clones induced with HMBA show little or no antisense globin RNA. Concomitant with the apparent reduction in antisense globin RNA upon HMBA induction, there is an increase in γ-β globin mRNA (Fig. 5). This 5- to 10-fold increase in γ-β globin mRNA upon induction contrasts sharply with the apparent disappearance of the antisense globin RNA that occurs simultaneously. Another probe representing a 360-bp fragment of the neo^R gene shows a slight reduction in the neo^R RNA level upon induction of MEL cells (data not shown). This indicates that

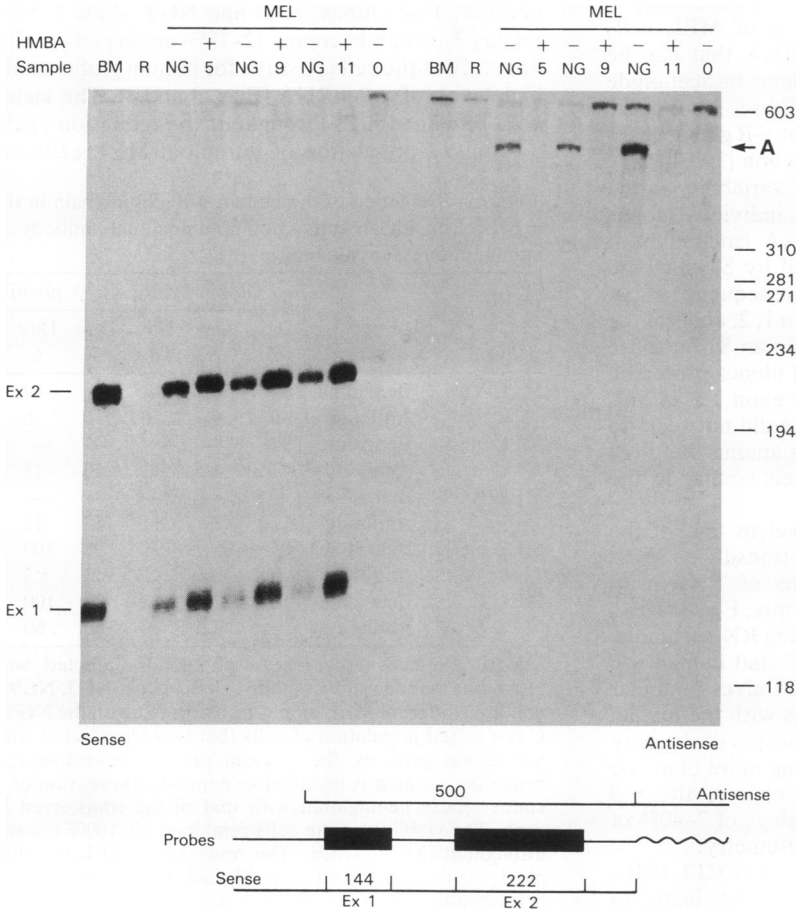

FIG. 5. (*Upper*) S1 nuclease analysis comparing levels of γ-β mRNA and antisense of γ-β globin RNA in three NG-transformed MEL clones (NG5, -9, and -11) with (+) or without (−) induction. The samples are the same as in Figs. 2 and 3 except the lane indicated by 0 represents RNA from MEL cells that have not been transformed with a retrovirus (negative control). All lanes have 20 μg of RNA except the BM lane that has 2 μg. (*Lower*) Probes used. The antisense probe (top line below the autoradiograph) was made from the opposite strand of the γ-β gene that was used to make the sense probe (bottom line). The nine samples on the left are analyzed using the sense probe. The right side of the figure shows the same samples analyzed with the antisense probe. The protected DNA probe reflecting the antisense fragment is present in all the uninduced NG-transformed clones as indicated by A (arrow). Methods are the same as in Fig. 2.

Table 2. Production of β-globin chains in MEL cells following retroviral gene transfer

Cell	Virus	Globin production, % positive cells
MEL diploid	β-2	46 (4–58)
MEL diploid	β-12	27.5
MEL tetraploid	β-2	78.5
MEL tetraploid	β-12	75.5 (43.5–95)

Globin production represents percent of positive MEL cells after immunofluorescent staining (25, 26) of multiclonal MEL cells infected with the β-globin viruses indicated. The range (in parentheses) indicates the number of positive cells in 24 subclones of the respective multiclonal population.

the viral genomic RNA levels are decreased during erythroid induction.

DISCUSSION

Retroviral vectors containing human genomic globin genes have been constructed and used to transfer these genes into MEL cells. The transferred genes were expressed at high levels in the form of both globin mRNA and globin protein. The amount of mRNA we observed was higher than that generated by a γ-β globin adenoviral vector transduced into K-562 cells (32) and those observed when the β-globin gene was transferred with retrovirus into MEL cells (18). Our observation of high levels of globin mRNA and the lack of inducibility of viral genomic RNA expression contrasts with the data obtained by Cone *et al.* (18). Considerable differences in the design of the retroviral vectors and functional differences between the MEL clones used might explain the contrasting results. Our data established the potential of using retroviral vectors to transfer and express globin genes at high levels in erythroid cells.

We provide evidence of, to our knowledge, the first well-documented production of globin protein achieved using the mechanism of retroviral-mediated globin gene transfer (Fig. 4, Table 2). The amount of the human β-globin protein synthesized in transduced MEL cells was at least 10% of the mouse globin level. These data contrast with the results obtained with the original vector containing a composite γ-β globin gene. A much lower frequency of positive cells was observed by immunofluorescence (Table 1), and very low levels of synthesis of the γ-β chain were observed (data not shown). These low levels of γ-β globin synthesis were observed in cells that were documented to contain high levels of the γ-β mRNA. Hence, we infer that the major factor leading to apparently low levels of protein synthesis with the γ-β construction is instability of the composite globin in MEL cells.

Both strands of the globin genes in our vectors are transcribed. The sense strand is transcribed from the globin promoter, whereas the antisense strand is part of the transcript originating from the viral long terminal repeat. We have indirect data indicating that the antisense RNA might contribute to the low amounts of γ-β chain synthesis. The same composite gene, when introduced into MEL cells by conventional DNA transfection, led to a much higher frequency of positive cells, as demonstrated by immunofluorescence. Sixty percent of such multiclonal tetraploid MEL cells were positive in contrast to 2–3% of multiclonal cells when the gene was introduced by a retroviral vector. Of interest is the fact that γ-β globin gene expression is lowest in clone NG11 that has the highest antisense/sense globin ratio (Figs. 4 and 5 and Table 1). The "disappearance" of detectable antisense globin RNA during erythroid induction (Fig. 5) could be due to formation of globin RNA duplexes. Most experiments done so far in eukaryotic cells have demonstrated antisense inhibition by supplying vast excesses of complementary RNA in *trans* (33, 34). A lower amount of antisense RNA might be needed to disturb function of complementary mRNA when both RNAs are transcribed at the same location. To determine the role of antisense RNA and its possible influence on protein expression will require more detailed studies using globin gene vectors that do not produce antisense RNA (18, 35, 36).

We thank Dr. A. D. Miller for valuable advice and provision of cell lines, Dr. R. C. Mulligan for the ψ2 cell line, Dr. E. Gilboa for the XM5 plasmid, and Dr. W. F. Anderson and associates and J. T. Holt for discussions and access to unpublished data. M. Brice, A. Cline, and B. Nakamoto provided expert technical assistance.

1. Varmus, H. E. (1982) *Science* **216**, 812–820.
2. Panganiban, A. T. (1985) *Cell* **42**, 5–6.
3. Mann, R. C., Mulligan, R. C. & Baltimore, D. (1983) *Cell* **33**, 153–159.
4. Miller, A. D., Trauber, D. R. & Buttimore, C. (1986) *Somatic Cell Mol. Genet.* **12**, 175–183.
5. Temin, H. M. (1986) in *Gene Transfer*, ed. Kucherlapati, R. (Plenum, New York), pp. 149–187.
6. Bernstein, A., Berger, S., Huszar, D. & Dick, J. (1985) in *Genetic Engineering*, eds. Setlow, J. K. & Hollaender, A. (Plenum, New York), Vol. 7, pp. 235–261.
7. Williams, D. A., Lemischka, I. R., Nathan, D. G. & Mulligan, R. C. (1984) *Nature (London)* **310**, 476–480.
8. Dick, J. E., Magli, M. C., Huszar, D., Phillips, R. A. & Bernstein, A. (1985) *Cell* **42**, 71–79.
9. Eglitis, M. A., Kantoff, P., Gilboa, E. & Anderson, W. F. (1985) *Science* **230**, 1395–1398.
10. Keller, G., Paige, C., Gilboa, E. & Wagner, E. F. (1985) *Nature (London)* **318**, 149–154.
11. Gruber, H. E., Finley, K. D., Hershber, R. M., Katzman, S. S., Laikind, P. K., Seegmiller, J. E., Friedman, T., Yee, J.-K. & Jolly, D. J. (1985) *Science* **230**, 1057–1061.
12. Hock, R. A. & Miller, A. D. (1986) *Nature (London)* **320**, 275–277.
13. Van der Putten, H., Botten, F. M., Miller, A. D., Rosenfeld, M. G., Fan, H., Evans, R. M. & Verma, I. M. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6148–6152.
14. Huszar, D., Balling, R., Kothary, R., Magli, M. C., Hozumi, N., Rossant, J. & Bernstein, A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8587–8591.
15. Jahner, D., Haase, K., Mulligan, R. & Jaenisch, R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6927–6931.
16. Anderson, W. F. (1984) *Science* **226**, 401–409.
17. Miller, A. D., Ong, E. S., Rosenfeld, M. G., Verma, I. M. & Evans, R. M. (1984) *Science* **225**, 993–998.
18. Cone, R. D., Weber-Benarous, A., Baorto, D. & Mulligan, R. C. (1987) *Mol. Cell. Biol.* **7**, 887–897.
19. Miller, A. D. & Buttimore, C. (1986) *Mol. Cell. Biol.* **6**, 2895–2902.
20. Karlsson, S., Humphries, R. K., Gluzman, Y. & Nienhuis, A. W. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 158–162.
21. Shimotohno, K. & Temin, H. M. (1982) *Nature (London)* **299**, 265–268.
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
23. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
24. Ley, T. J., Anagnou, N. P., Pepe, G. & Nienhuis, A. W. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4975–4979.
25. Stamatoyannopoulos, G., Farquhar, M., Lindsley, D., Brice, M., Papayannopoulou, Th. & Nute, P. E. (1983) *Blood* **61**, 530–539.
26. Papayannopoulou, Th., Lindsley, D., Kurachi, S., Lewison, K., Hemenway, T., Melis, M., Anagnou, N. P., Najfeld, V. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 780–784.
27. Marks, P. A. & Rifkind, T. A. (1978) *Annu. Rev. Biochem.* **47**, 419–426.
28. Karlsson, S. & Nienhuis, A. W. (1985) *Annu. Rev. Biochem.* **54**, 1071–1108.
29. Wright, S., Rosenthal A., Flavell, R. A. & Grosveld, F. G. (1984) *Cell* **38**, 265–273.
30. Anagnou, N. P., Karlsson, S., Moulton, A. D., Keller, G. & Nienhuis, A. W. (1986) *EMBO J.* **5**, 121–126.
31. Tsapis, A., Hinard, N., Testa, J., Dubart, A., Vainchenker, W., Rouyer-Fessard, Ph., Beuzard, T., Rosa, J. (1980) *Eur. J. Biochem.* **112**, 513–519.
32. Karlsson, S., Van Doren, K., Schweiger, S. G., Nienhuis, A. W. & Gluzman, Y. (1986) *EMBO J.* **5**, 2377–2385.
33. Izant, J. G. & Weinbraub, H. (1985) *Science* **229**, 345–352.
34. Kim, S. K. & Wold, B. J. (1985) *Cell* **42**, 129–138.
35. Sorge, J., Wright, D., Erdman, V. D. & Cubbing, A. E. (1984) *Mol. Cell. Biol.* **4**, 1730–1737.
36. Yu, S.-F., von Ruden, T., Kantoff, P. W., Garber, C., Seiberg, M., Ruther, U., Anderson, W. F., Wagner, E. F. & Gilboa, E. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 3194–3198.