The EMBO Journal Vol. 1 No. 1, pp.15–20, 1982

Transfer of human globin genes to erythroleukemic mouse cells

D.A.Spandidos and J.Paul*

Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow, G61 1BD, UK

Communicated by J.Paul
Received on 11 November 1981

Thymidine kinase negative (TK$^-$) Friend cells were transformed with recombinant molecules carrying human globin genes and the thymidine kinase gene of herpes simplex virus type 1 DNA. Transformation frequencies of 1 transformant/μg donor DNA/1 x 10^6 cells were obtained by standard procedures and this was increased 20- to 30-fold by treating recipient cells with dimethyl sulfoxide or glycerol. Transformed cell lines expressed thymidine kinase activity of viral origin as determined by its insensitivity to 0.2 mM dTTP and electrophoretic mobility in polyacrylamide gels. The physical status of donor DNA in the transformed cells was examined in Hirt precipitates and supernatants by Southern blot hybridization and spot hybridization techniques. This analysis showed that most donor sequences were present in a circular or concatenate configuration, but also was suggestive of some donor sequences being integrated into high molecular weight DNA. Expression of human globin genes and particularly the ϵ-globin gene in the transformed Friend cells was studied by Northern blot hybridization analysis.

Key words: erythroleukemic cells/gene transfer/globin genes/herpes thymidine kinase

Introduction

The nucleotide sequence of the ϵ, $^G\gamma$, $^A\gamma$, δ, β, a1 and a2 structural globin genes has been completed and detailed maps of restriction enzyme sites in the extragenic regions are available (Efstratiadis *et al.*, 1980; Liebhaber *et al.*, 1981). The structural studies by themselves have thrown little light on gene expression and regulation during growth and development but gene transfer methods combined with site-directed mutagenesis should make it possible to identify sequences involved in the regulation of human globin gene expression.

Cellular genes such as globin (Mantei *et al.*, 1979; Wold *et al.*, 1979) and ovalbumin (Lai *et al.*, 1980) which are expressed in differentiated cells, have been introduced into mouse L cells but, since L cells do not normally express these genes, the physiological significance of the experiments is dubious. It might be more informative to introduce a globin gene into a cell which normally expresses globin genes, especially if expression could be modulated by an appropriate inducer. The Friend erythroleukaemic cell fulfils these criteria.

We have therefore, studied the transfer of the cloned herpes simplex virus (HSV) thymidine kinase (TK) gene covalently linked to human globin genes in TK$^-$ Friend cells. We optimized conditions for efficient gene transfer with a particular Friend cell line, then examined the physical status of donor DNA within the host and, finally, studied the expression of the transferred human ϵ globin gene in induced and non-induced transformed Friend cells.

Results

Transformation of Friend cells with human globin recombinants carrying the TK gene of HSV-1 DNA

We first constructed recombinant molecules carrying genomic DNA fragments coding for the β, δ, ϵ and a1 globin genes and the TK gene of HSV-1 DNA. The restriction maps and the construction of these recombinant plasmids is described in the legends of Figures 1 and 2. Plasmid pMX was constructed by P.Montague who also collaborated in the construction of plasmids pTKHϵG-1 and pTKHϵG-2. Figure 3 shows a BamHI restriction digest of some of these plasmids.

We have developed a methocel assay for transformation of TK deficient mouse (LMTK$^-$) and hamster (BHKTK$^-$) cells with HSV TK recombinants, and have shown that no DNA carrier is required for efficient transformation. Moreover, since linearized recombinant molecules gave the same frequency of transformation as closed circular molecules, recovered after banding in CsCl gradients in the presence of ethidium bromide, closed circular molecules were used in the studies described here.

We attempted to transform six different TK$^-$ Friend erythroleukemic cell lines with one or more of the recombinant molecules described in Figure 1. The reversion frequencies and transformation frequencies of these cell lines are shown in Table I. Ten clones of each cell line which had reacquired TK activity were examined by TK assay in the presence of dTTP and by spot hybridization with nick-translated [^{32}P] pTK-1 DNA to determine expression of HSV TK activity and the presence of donor DNA sequences. Only two out of 10 clones of the transformed 707B10/1 cells were found to express HSV TK activity and to carry pTK-1 donor sequences. The other eight expressed TK activity of cellular type and were therefore revertants. All clones tested from cell lines F4-B8/4, F4N + 2B4, F4 + BC1 and F4-B8/3B were negative for HSV TK expression and pTK-1 sequences. However, all 10 clones obtained with the F4-12B2 cell line were found to be positive for both expression of HSV TK and presence of pTK-1 donor sequences (see below), and this has proved to be true of all potentially transformed F4-12B2 cell clones examined so far. Similar results have been obtained by P. Montague in this laboratory in experiments with recombinant pMX and deletion mutants of this vector. As shown in Table I the reversion frequency of the F4-12B2 TK$^-$ cell line is very low.

Optimisation of the transformation frequencies of F4-12B2 cells with dimethylsulphoxide and glycerol

Various facilitators are known to increase viral infectivity or transformation frequency (Stow and Wilkie, 1976). We have, therefore, examined whether the known facilitators dimethylsulphoxide (DMSO) or glycerol, could increase transformation frequency in this system. Results with the recombinant pTKH$\beta\alpha$G-10 are shown in Figure 4. DMSO produced the greatest enhancement of transformation at a

*To whom reprint requests should be sent.

 0261-4189/82/0101-0015$2.00/0

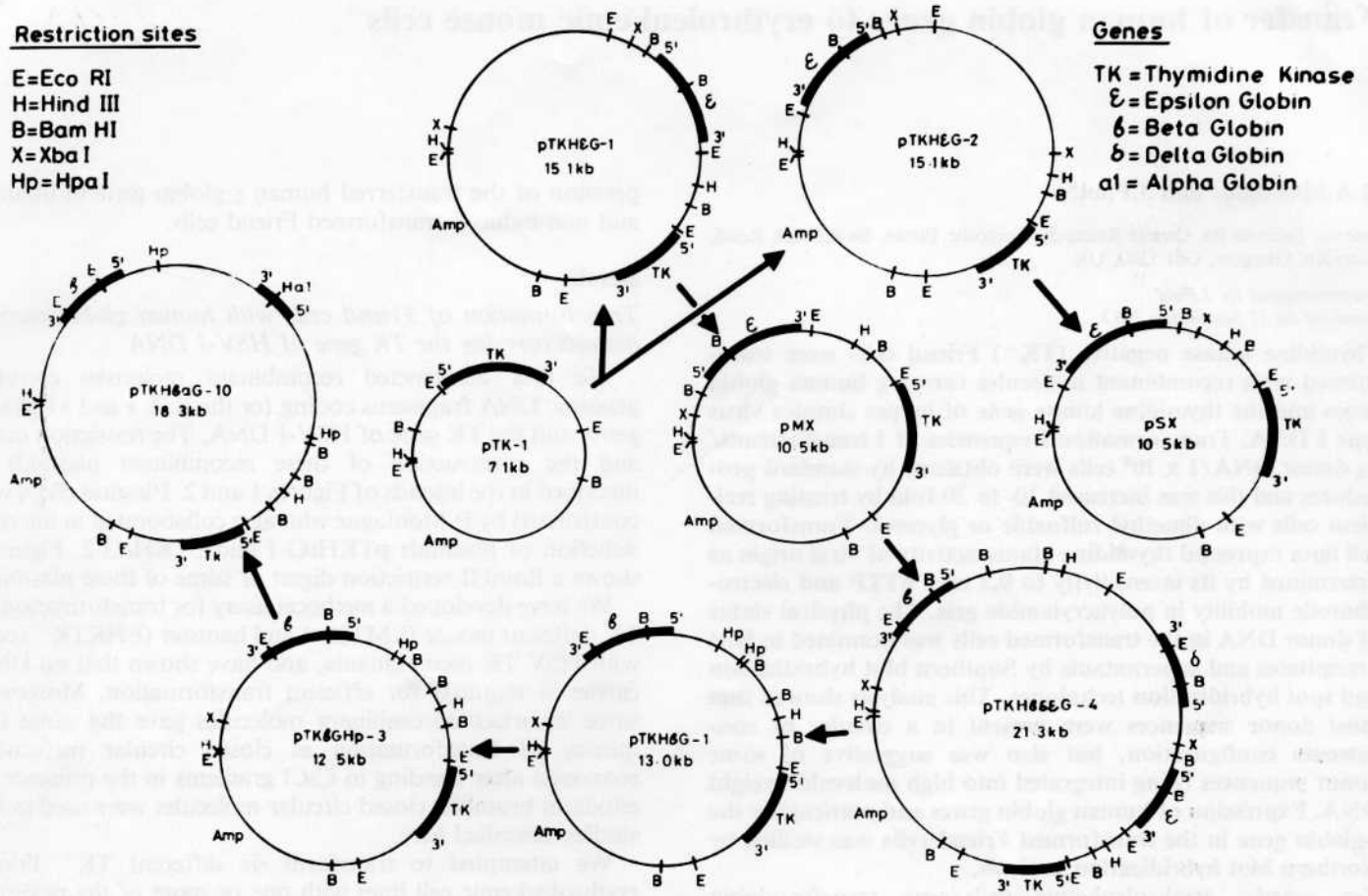

Fig. 1. Construction of recombinant plasmids used in gene transfer experiments. The plasmid pTK-1 (Wilkie *et al.*, 1979) was constructed by inserting a 3.5 kb HSV-1 DNA fragment carrying the TK gene into the single BamHI site of plasmid pAT-153 (Twigg and Sherratt, 1980). **A**, recombinant plasmids pTKHεG-1 and pTKHεG-2 carrying the human embryonic ε globin gene were constructed by inserting a HindIII 8.0 kb human DNA fragment of the recombinant phage 788 (Proudfood and Baralle, 1979) into the single HindIII site of pTK-1 in both orientations. **B**, plasmid pMX was constructed by deleting the 4.6 kb fragment between the two XbaI sites of plasmid pTKHεG-1. **C**, plasmid pSX was obtained in the same way from plasmid pTKHεG-2. **D**, plasmid pTKHβδεG-4 was constructed by inserting a 10.8 kb XbaI fragment of the recombinant phage λ HβG2 (Lawn *et al.*, 1978) containing the human β and δ genes into the single XbaI site of plasmid pMX. pTKHβδεG-4 contains the intact human β, δ and ε globin genes. **E**, plasmid pTKHβG-1 was constructed by partial digestion of pTKHβδεG-4 with HindIII and ligation. δ and ε globin genes are lost and the β globin gene is covalently linked to the TK gene. **F**, plasmid pTKHβGHp-3 was constructed by digestion of plasmid pTKHβG-1 with HpaI and ligation. An 0.5 kb HpaI DNA fragment has been deleted 800 bp upstream from the 5′ end of the β globin gene thus generating plasmid pTKHβGHp-3 which carries a single HpaI site. **G**, plasmid pTKHβαG-10 was constructed by inserting a 5.8 kb DNA fragment of the recombinant phage HaG2 (Lauer *et al.*, 1980) into the single HpaI site of pTKHβGhp-3. pTKHβαG-10 contains the intact human β and a1 globin genes.

concentration of 5% after 4 min treatment of the cells at room temperature. Above this concentration transformation was reduced drastically and this was accompanied by reduction of plating efficiency (unpublished results). The optimum concentration of glycerol was 10%. Under optimum concentrations both DMSO and glycerol could increase the transformation frequency by a factor of 20–30.

TK activity in transformed Friend cells

Two different assays were used to measure TK activity in transformed cells. First the TK activity of cell extracts was determined by measuring the conversion of [^{3}H]TdR to [^{3}H]-thymidine phosphates in the presence of 0.2 mM dTTP (Jamieson and Subak-Sharpe, 1974); this compound inhibits wild-type Friend cell TK activity >95% (unpublished results). As shown in Figure 5, non wild-type TK activity measured in transformed F4-12B2TK$^+$ cells is 50–100 times higher than in recipient F4-12B2TK$^-$ cells. Similar results were found with 24 other F4-12B2TK$^+$ clones.

Further evidence for the presence of HSV-type TK was obtained in the transformed 707B10/1 clone T-14 by examining the electrophoretic profiles of cytosol fractions derived from

Table I. TK$^-$ Friend erythroleukemic mouse cells used as recipients in gene transfer experiments[a].

Cell line[b]	Reversion frequency[c]	Transformation frequency[d]
707 B10/1	1×10^{-7}	5×10^{-8}
F4-B8/4	1×10^{-5}	–
F4N + 2B4	6×10^{-5}	–
F4 + BC1	3×10^{-6}	–
F4-B8/3B	4×10^{-5}	–
F4-12B2	1×10^{-9}	1×10^{-6}

[a]Gene transfer was carried out as described in **Materials and methods.**

[b]707 B10/1 cells grow in suspension whereas all the others grow in monolayers with 60–80% of cells attached to the flask.

[c]Determined by plating in HAT medium containing 0.9% methocel.

[d]Determined after testing the cells from colonies obtained in HAT medium containing methocel for HSV TK activity and pTK-1 sequences by spot hybridization analysis as described in **Materials and methods.**

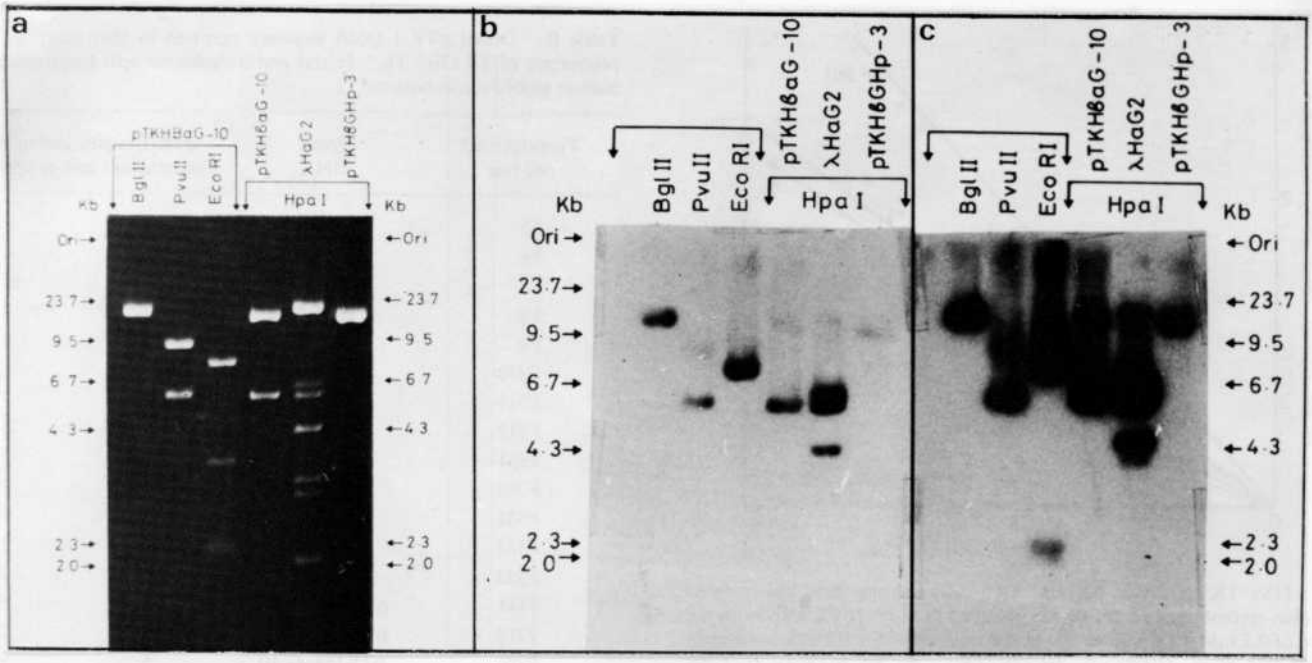

Fig. 2. Construction of the pTKHβαG-10 recombinant molecule. The λ HaG2 recombinant phage (Lauer *et al.*, 1980) was grown and purified in CsCl gradients. Two bands of phage particles were seen as described, a prominent upper band containing two deletion types of phage recombinants (leftward and rightward deletions) and a fainter lower band containing wild-type recombinant. The upper band was collected and DNA extracted and digested with HpaI. The HpaI fragments were subcloned into the single HpaI site of pTKHβGHp-3 as described in **Materials and methods.** Recombinants carrying human globin genes were screened as follows: DNAs from 38 colonies were alkaline-extracted (Birnboim and Doly, 1979), digested with HpaI, analysed on 1% agarose, and transferred to nitrocellulose filters (Southern, 1975). HpaI digests of λ HaG2 DNA were run in parallel as markers. The filters were hybridized with a [^{32}P]cDNA probe made against mRNA from fetal exchange blood, containing 50% α globin mRNA. Two out of 38 colonies were found to carry human α globin DNA. One of them, recombinant pTKHβαG-10, was grown and analysed in more detail. As shown in **a** pTKHβαG-10 was digested with BglII, PvuII, EcoRI or HpaI and run in 1% agarose gel in parallel with digests of λ HaG2 and pTKHβGHp-3 with HpaI. The DNA was transferred to nitrocellulose and hybridized with a [^{32}P]cDNA probe made against RNA from fetal exchange blood. A short exposure is shown in **b** and a longer one in **c**. The hybridization pattern is consistent with the restriction map of Figure 1. Further restriction enzyme digests (not shown) have confirmed that the HpaI fragment of λ HaG2 transferred to the HpaI site of pTKHβGHp-3 contains the intact a1 human globin gene.

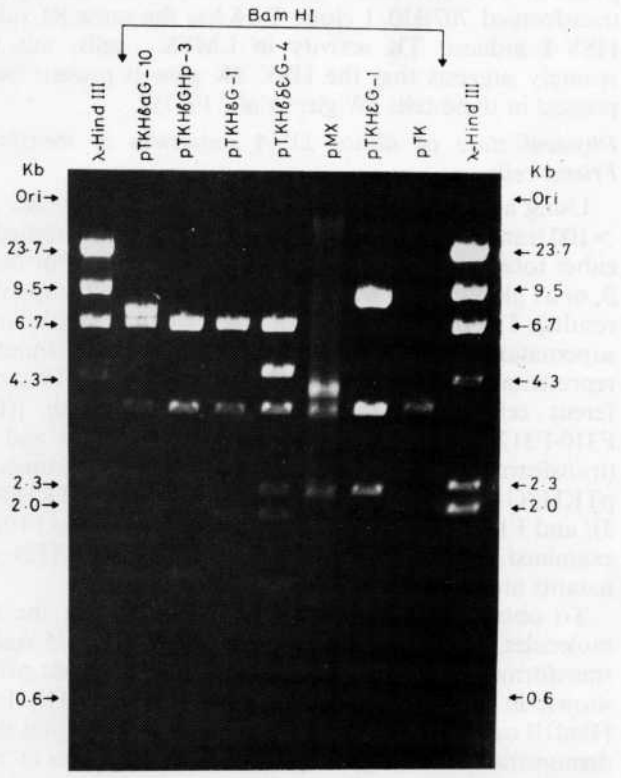

Fig. 3. Digestion of human globin recombinant plasmids with BamHI and electrophoretic analysis of the resulting DNA fragments on 1% agarose gel. HindIII digests of λ DNA were used as molecular weight markers.

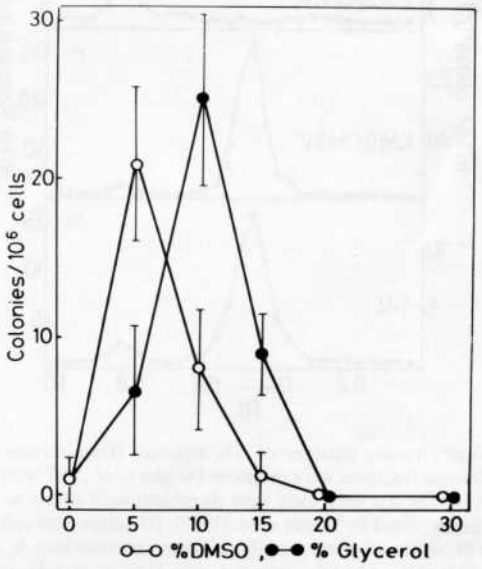

Fig. 4. Effect of DMSO and glycerol concentrations on the enhancement of pTKHβαG-10 DNA transformation of F4-12B2 TK$^-$ erythroleukemic cells. Following incubation for 4 h at 37°C with the calcium phosphate-DNA precipitate the medium was removed and 2 ml of PBS containing the various concentrations of DMSO or glycerol were added to each 75 cm^2 flask subconfluent with F4-12B2 TK$^-$ cells. After 4 min at room temperature the DMSO- or glycerol-containing PBS was removed and the cells were washed with 10 ml of PBS. SF12 medium containing 15% FCS was added and incubation continued at 37°C for 20 h. Cells were then trypsinized, counted and plated in HAT medium containing 0.9% methocel. Colonies were counted and picked 8 days later. Each point represents the average number of colonies from four flasks and the error bars represent ± 2 s.d.

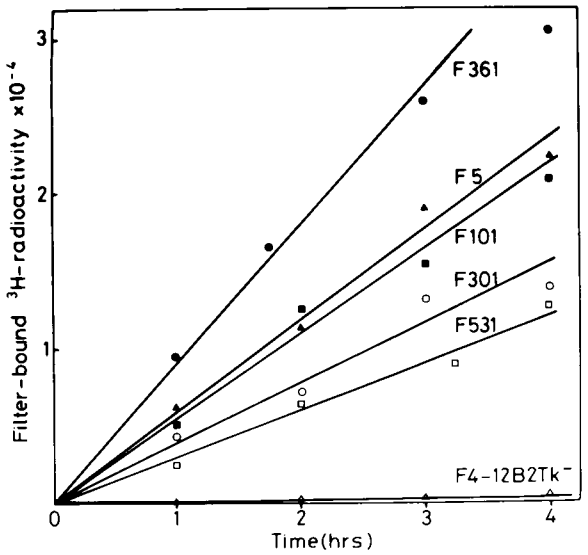

Fig. 5. HSV TK activity in F4-12B2 TK$^-$ cells and the same transformed with globin recombinants carrying the TK gene of HSV-1. TK activity in the presence of 0.2 mM TTP was determined in extracts of 5 x 10^5 cells as described by Wilkie *et al.* (1979). △, F4-12B2TK$^-$; ▲, F5 (pTK-1); 0, F301 (pTKHεG-1); ●, F361 (pTKHεG-2); □, F531 (pTKHβG-1); and ■, F101 (pTKHβαG-10).

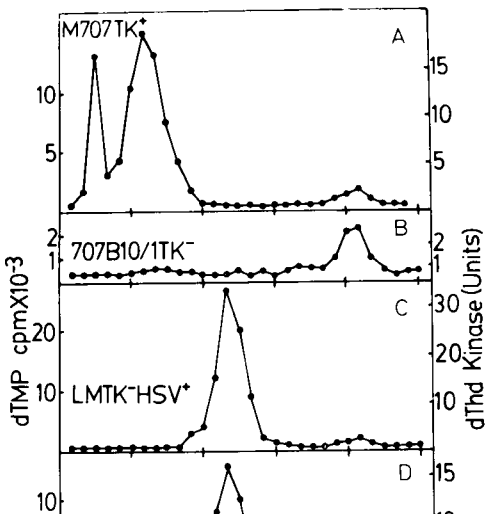

Fig. 6. Electrophoretic patterns of TK activities from various Friend cell lines. Cytoplasmic fractions were prepared (Wigler *et al.*, 1977) and applied to 5% polyacrylamide disc gels. Gels were sliced and each slice was assayed for TK-activity as described by Wigler *et al.* (1977). Rf values were calculated with reference to the electrophoretic mobility of bromophenol blue **A**, M707TK$^+$; **B**, 707B10/1TK$^-$; **C**, LMTK$^-$ infected with HSV-1; and **D**, transformant T-14, a 707B10/1TK$^-$ cell line transformed to TK$^+$ with HSV-1 DNA digested with BamH1.

the cell line and, as controls, M707TK$^+$ (a wild-type Friend cell line from which the 707B10/1 was derived) and the recipient 707B10/TK$^-$ and LMTK$^-$ cells infected with HSV. As shown in Figure 6, M707TK$^+$ showed three peaks of TK activity with Rf values at 0.1, 0.25 and 0.85, whereas 707B10/1TK$^-$ showed only one at 0.85 (mitochondrial TK). Clone T14 and LMTK$^-$ infected with HSV-1 showed two

Table II. Donor pTK-1 DNA sequence numbers in Hirt supernatant and precipitate of F4-12B2 TK$^+$ Friend erythroleukemic cells transformed with human globin recombinants[a].

Transformed cell line	Donor DNA	pTK-1 copies/cell in Hirt supernatant and precipitate	
F5	pTK-1	12	8
F6	pTK-1	8	4
F7	pTK-1	13	10
F8	pTK-1	2	2
F9	pTK-1	3	9
F310	pTKHεG-1	9	1
F311	pTKHεG-1	9	7
F312	pTKHεG-1	38	6
F361	pTKHεG-2	11	7
F362	pTKHεG-2	9	7
F531	pTKHβG-1	8	3
F532	pTKHβG-1	18	2
F533	pTKHβG-1	11	2
FH1	pTKHβGHp-3	1	6
FH2	pTKHβGHp-3	2	2
F101	pTKHβαG-10	5	9
F102	pTKHβαG-10	2	8
F103	pTKHβαG-10	1	7
F104	pTKHβαG-10	19	2
F105	pTKHβαG-10	8	2

[a]After autoradiography each spot was cut out and ^{32}P was counted by liquid scintillation. The average of duplicate samples was taken to estimate the number of pTK-1 DNA equivalent copies/cell. The assumption was made that each cell contains 10 pg DNA.

peaks at 0.45 and 0.85. Since the major TK activity in the transformed 707B10/1 clone T-14 has the same Rf value as HSV-1 induced TK activity in LMTK$^-$ cells, this result strongly suggests that the HSV TK gene is present and expressed in these cells (Wigler *et al.*, 1977).

Physical state of donor DNA sequences in transformed Friend cells

Using a spot hybridization assay (Spandidos *et al.*, 1981) >100 transformed F4-12B2TK$^+$ cell lines were screened with either total recombinant DNA or specific probes for the ε, δ, β, or a1 globin genes which we have constructed (unpublished results). The presence of donor DNA was also studied in Hirt supernatants and precipitates of various transformants. A representative study is summarised in Table II. Twenty different cell lines F5 to F9 (transformed with pTK-1), F310-F312 (transformed with pTKHεG-1), F361 and F362 (transformed with pTKHεG-2), F531-F333 (transformed with pTKHβG-1), FH1 and FH2 (transformed with pTKHβGHp-3), and F101 to F105 (transformed with pTKHβαG-10) were examined. Donor sequences were found in both Hirt supernatants and precipitates.

To obtain information about the integrity of the donor molecules, Southern blots of total DNA from 35 cell lines transformed with one or other of the recombinant plasmids shown in Figure 1 were analysed, using BamHI, EcoRI, HindIII or HpaI and various probes. The results (not shown) demonstrated full length copies of donor sequences in >90% of the transformed cells. Similar analyses (except for HpaI) were applied to the Hirt supernatants and precipitates of the following eight cell lines; F5 and F8 (transformed with pTK

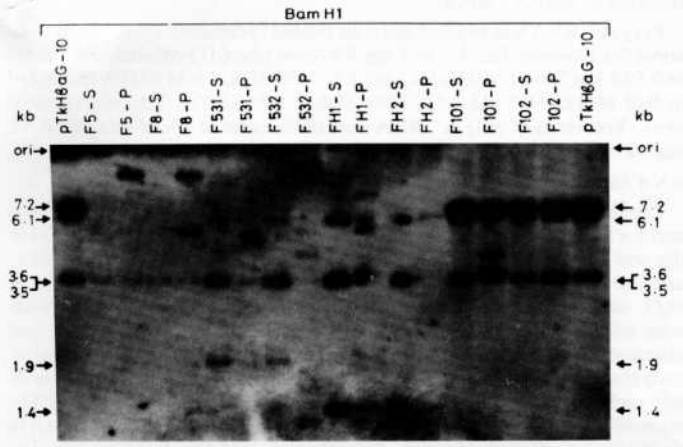

Fig. 7. Southern blot analysis of the DNA isolated from F4-12B2TK$^+$ mouse erythroleukemic Friend cells transformed with globin recombinants. DNA was isolated from the Hirt precipitates and supernatants, digested with BamHI, electrophoresed on 1% agarose, transferred to nitrocellulose filters and hybridized with the ^{32}P-labelled nick-translated pTKH$\beta\alpha$G-10 DNA. 200 pg of pTKH$\beta\alpha$G-10 DNA, on a background of 10 μg total F4-12B2TK$^-$ cell DNA was digested with BamHI and run in parallel (first and last well in the gel) to serve as a marker. Friend cells were transformed with pTK-1 (F5 and F8), pTKHβG-1 (F531 and F532), pTKHβGHp-3 (FH1 and FH2), and pTKH$\beta\alpha$G-10 (F101 and F102); S, Hirt supernatant; P, Hirt precipitate. 10 μg DNA from each precipitate was used in each digest and the corresponding amount of DNA from the Hirt supernatant obtained from an equivalent number of cells.

1); F531 and F532 (transformed with pTKHβG-1); FH1 and FH2 (transformed with pTKHβGHp-3); and F101 and F102 (transformed with pTKH$\beta\alpha$G-10). The BamHI result (Figure 7) shows full length copies of donor sequences in all fractions. Extra bands in the digests from Hirt precipitates could arise from copies integrated into chromosomal DNA or from rearranged plasmids.

Expression of human gene in transformed Friend cells

The presence of transcripts from donor DNA sequences in transformed Friend cells was studied by Northern blot and spot hybridization analyses. With a human β globin-specific probe, 9S mRNA was demonstrated in cells transformed with pTKH$\beta\delta\epsilon$G-4, pTKHβG-1, pTKHβGHp-3, and pTKH$\beta\alpha$G-10. Human α globin-specific 9S RNA was also found in cells transformed with pTKH$\beta\alpha$G10 (results not shown). In more detailed studies, three independently isolated Friend cell lines transformed with pTKHϵG-1 molecules were examined. Cells were treated with the inducer (3 mM hexamethylene bis-acetamide (HMBA) in hypoxanthine/aminopterin/thymidine (HAT) medium for 6 days or left untreated in HAT medium for a similar period. Poly A$^+$ RNA was then isolated from the total RNA from each transformed cell line. After electrophoresis and Northern blot hybridization analysis using a probe containing the 3′ end of the human ϵ globin gene, 9S transcripts could be shown (Figure 8). RNA prepared from K562 cells which contains human ϵ globin mRNA (Benz *et al.*, 1980; Cioe *et al.*, 1981; Rutherford *et al.*, 1981) was used as a marker. RNA from the same cells was also probed with TK DNA, pAT153 DNA and mouse β globin DNA using the spot hybridization assay (Spandidos *et al.*, 1981); transcripts from all these sequences were found. Expression was variable in different cultures and, although in the experiment illustrated there was an apparent correlation between relatively high concentrations of ϵ globin mRNA and treatment of transformed Friend cells with HMBA, this phenomenon has not been consistently observed.

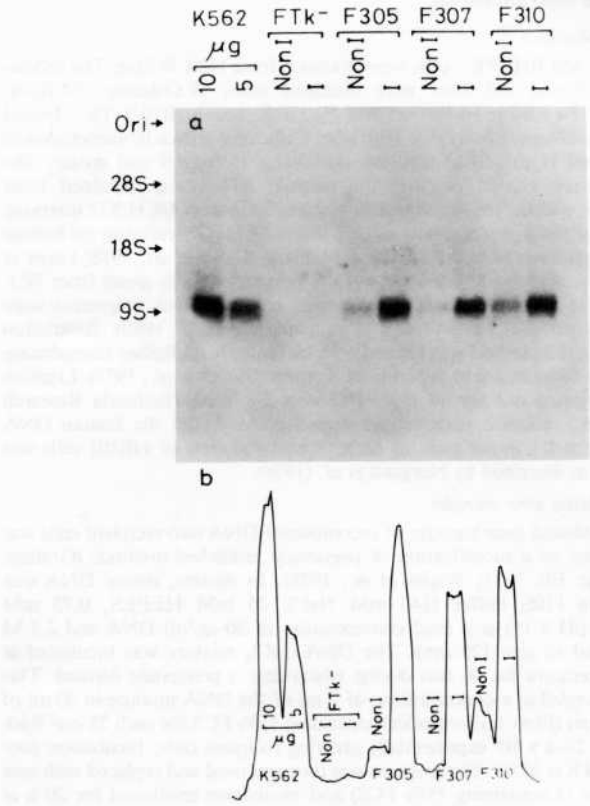

Fig. 8. Northern blot hybridization of poly A$^+$ RNA isolated from induced and non-induced F4-12B2TK$^+$ cells transformed with pTKHϵG-1 DNA. Ten and 5 μg respectively of total RNA isolated from uninduced K562 cells served as a marker. Poly A$^+$ RNA was isolated from 200 μg total RNA of recipient (F4-12B2TK$^-$ (FTK$^-$)) and transformed (F305, F307 and F310) cells uninduced and induced with 3 mM HMBA cells. The BamHI–EcoRI 1.3 kb DNA fragment containing the 3′ region of the human ϵ globin gene and extragenic region was labelled with ^{32}P by nick-translation and used as a probe. **a**, Autoradiogram. **b**, Densitometer scanning profile.

Discussion

The successful transfer of plasmids to Friend cells was entirely dependent on identifying a suitable host cell line. Not only was it necessary to find a TK$^-$ cell in which the reversion frequency of the mutation was low but there was also great variation among different cell lines in their competence to be transformed by recombinant DNA. The reasons for this are not clear but the line F4-12B2 is the most adherent (80–90%) of the adherent cell lines tested and the 707B10/1 cell line grows only in suspension.

In these experiments, in which closed circular DNA molecules were used without carrier, most of the transferred molecules were found intact within the host cell. It seems likely that the copies in the Hirt supernatant are either free circles or concatenates similar to "pekelasomes" (Perucho *et al.*, 1980). The significance of the few copies in the Hirt precipitate is difficult to interpret for, at this low copy number, they might represent contamination from the supernatant. However, the Southern analyses would support the idea that some copies are integrated into chromosomal DNA. In all cases the transformed cells carried several copies of transferred DNA which must, therefore, be replicated.

The ability to introduce cloned human globin genes into erythroid mouse cells as demonstrated in this study is potentially a powerful tool for studying the regulation of gene expression and for identifying DNA sequences which might be responsible.

Materials and methods

Cells and plasmids

LMTK$^-$ and BHKTK$^-$ cells were obtained from N.M.Wilkie. The following TK$^-$ Friend cell lines were obtained from W.Ostertag: F4-B8/4; F4N + 2B4; F4 + BC1; F4-B8/3B; and F4-12B2. The 707B10/1 TK$^-$ Friend cell line was obtained from P.R.Harrison. Cells were grown in monolayers in supplemented Ham's SF12 medium containing 15% fetal calf serum. The bacterial strain HB101 carrying the plasmid pTK-1 was obtained from N.M.Wilkie (1979). The recombinant phages, λ Charon 4A HβG2 (carrying the human β and δ globin genes) and λ Charon 4AHαG2 (carrying the human a1 globin gene) were obtained from T.Maniatis (Lawn *et al.*, 1978; Lauer *et al.*, 1980), and phage λ 788 (carrying the human ε globin gene) from N.J. Proudfoot and F.E.Baralle (1979). Human genomic DNA fragments were recloned in pBR322 or pAT-153 (Twigg and Sherratt, 1980). Restriction enzyme-digested plasmid was treated with calf intestinal alkaline phosphatase (Boehringer Mannheim) to prevent self-ligation (Ullrich *et al.*, 1977). Ligation was then carried out for 18 h at 4°C with T4 ligase (Bethesda Research Laboratories), alkaline phosphatase-treated plasmid and the human DNA fragment at a 1:1 molar ratio of each. Transformation of HB101 cells was performed as described by Norgard *et al.* (1978).

DNA-mediated gene transfer

DNA-mediated gene transfer of recombinant DNA into recipient cells was accomplished by a modification of previously published methods (Graham and van der Eb, 1973; Wigler *et al.*, 1978). In outline, donor DNA was dissolved in HBS buffer (140 mM NaCl, 25 mM HEPES, 0.75 mM Na_2HPO_4, pH 7.12) at a final concentration of 20 μg/ml DNA and 2.5 M $CaCl_2$ added to give 125 mM. The DNA-$CaCl_2$ mixture was incubated at room temperature for 30 min during which time a precipitate formed. This was resuspended at a concentration of 2 ml of the DNA mixture in 20 ml of SF12 medium (Flow Laboratories) containing 15% FCS for each 75 cm^2 flask containing 2–4 x 10^6 exponentially growing recipient cells. Incubation continued for 4 h at 37°C. The medium was then removed and replaced with new medium (SF12 containing 15% FCS) and incubation continued for 20 h at 37°C. Cells were trypsinized, counted and plated in HAT medium (SF12 containing 30% FCS, 0.9% methocel (Dow Chemicals), 100 μM hypoxanthine, 0.8 μM aminopterin and 15 μM thymidine). Colonies were picked 7–10 days later with a Pasteur pipette and grown into SF12 medium containing 15% FCS and HAT. The non-adherent 707B10/1 TK$^-$ cells were exposed to the calcium phosphate-DNA coprecipitate while in suspension. 2–4 x 10^6 cells were resuspended in the transformation mixture (2 ml) and incubated at room temperature for 30 min. Twenty ml of medium were added to the mixture and the cells were further incubated at 37°C for 4 h. The medium was removed and replaced with new medium as above and incubation continued for 20 h at 38°C. Cells were then harvested, counted and plated in HAT medium as above.

Electrophoresis and filter hybridization

The restriction endonucleases were purchased from BRL and DNA was digested using conditions suggested by BRL. Electrophoresis on agarose gels and filter hybridizations were performed as described by Wahl *et al.* (1979). DNA was labelled by nick-translation using ^{32}P-labelled nucleotide triphosphates (Amersham) as described by Rigby *et al.* (1977).

Assay of TK activity

The assay of TK activity in the presence of 0.2 mM dTTP in recipient and transformed cell lines was performed as described by Wilkie *et al.* (1979). Polyacrylamide gel electrophoresis analyses were performed as previously described (Lee and Cheng, 1976; Wigler *et al.*, 1977).

Isolation of DNA

Plasmid DNA was isolated as described by Wilkie *et al.* (1979). High molecular weight DNA from recipient and transformant cells was obtained as described by Gross-Bellard *et al.* (1973). Hirt extraction of cell lines was performed as described by Hirt (1967).

RNA extraction from cells

Cells were trypsinized, washed with PBS and resuspended in guanidine-HCl buffer (8.0 M guanidine-HCl, 20 mM sodium acetate, 50 mM EDTA, 5% β-mercaptoethanol, pH 7.0) at a concentration of 0.5–1 x 10^8 cells/ml. Cell lysates were homogenized in a Dounce homogenizer (8–10 strokes), made 2% with SDS and heated at 65°C for 2–3 min. After vortexing, 5 ml of cell lysate were placed on a 3 ml cushion of CsCl solution (5.7 M CsCl, 50 mM EDTA) and centrifuged for 48 h at 40 K, 15°C in a 10 x 10 ml Ti rotor. The RNA pellet was resuspended in 2.0 M LiCl, 4.0 M urea and left at 4°C overnight in a corex tube. RNA was pelleted at 10 K r.p.m. for 15 min in a Sorvall centrifuge, resuspended in 0.1 x MOPS buffer (1 x MOPS + 20 mM Na MOPS, 5 mM sodium acetate, 1 mM EDTA, pH 7.0) and dialysed in the same buffer for 2 h before lyophilising. Recoveries were of the order of 1 mg RNA/10^8 cells.

Isolation of poly A$^+$ RNA

Poly A$^+$ RNA was isolated using an oligo(dT)-cellulose Type 3 from Collaborative Research Inc. Up to 1 mg RNA/ml oligo(dT)-cellulose was loaded in 0.5 M NaCl, 0.01 M Tris-HCl pH 7.5, 0.5% SDS, 1 mM EDTA and eluted in 0.01 M Tris-HCl pH 7.5, 0.05% SDS, 1 mM EDTA. This was repeated twice. Recoveries of poly A$^+$ RNA were of the order of 5% of total cell RNA loaded on the column.

RNA blotting on to nitrocellulose

Lyophilysed RNA was taken up in 50% formamide, 2.2 M formaldehyde and 1 x MOPS buffer, heated at 55°C for 15 min, made 10% glycerol, 0.1% dye and layered on to a gel. The gel containing 1% agarose, 1 x MOPS buffer, and 2.2 M formaldehyde was made as follows: the agarose was melted in H_2O, cooled to 60°C and the 10 x MOPS buffer and 13.2 M formaldehyde were added. The gel was run for 3–4 h at 3 V/cm until marker dye had migrated 8–10 cm. It was then soaked in 10 volumes of 1 x MOPS buffer (two changes of 15 min each) and subsequently in 20 x SSC (two changes of 30 min each). RNA was blotted on to nitrocellulose in 20 x SSC for 4–6 h. The nitrocellulose filter was washed in 3 x SSC, air dried and baked for 3–4 h in an 80°C oven. Prehybridization, hybridization, and filter washing were performed as described for Southern blotting analysis.

DNA and RNA spot hybridization procedures were carried out as described in Spandidos *et al.* (1981).

Acknowledgements

We are grateful to Dr. P.R.Harrison and Mr. P.Montague for discussions and checking some findings. D.A.S. was a Canadian MRC Centennial Fellow. The research was supported by grants from the MRC and CRC.

References

Benz,E.J., Murnane,M.J., Tonkonow,B.L., Berman,B.W., Marur,E.M., Cavallesco,G., Jenko,T., Snyder,E.L., Forget,B.G., and Hoffman,R. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 3509-3513.

Birnboim,H.C., and Doly,J. (1979) *Nucleic Acids Res.*, **7**, 1513-1523.

Cioe,L., McNab,A., Hubbell,H.R., Meo,P., Curtis,P., and Rovera,G. (1981) *Cancer Res.*, **41**, 237-243.

Efstratiadis,A., Posakony,J.W., Maniatis,T., Lawn,R.M., O'Connell,C., Spritz,R.A., Deriel,J.K., Forget,B.G., Weissman,S.M., Slighton,J.L., Blechl,A.E., Smithies,O., Baralle,F.E., Shoulders,C.C., and Proudfoot, N.J. (1980) *Cell*, **21**, 653-688.

Graham,F.L., and van der Eb,A.J. (1973) *Virology*, **54**, 536-539.

Gross-Bellard,M., Oudet,P., and Chambon,P. (1973) *Eur. J. Biochem.*, **36**, 32-36.

Hirt,B. (1967) *J. Mol. Biol.*, **26**, 365-369.

Jamieson,A.T., and Subak-Sharpe,J.H. (1974) *J. Gen. Virol.*, **24**, 481-492.

Lai,E.C., Woo,S.L.C., Brodelon-Riser,M.E., Fraser,T.H., and O'Malley, B.W. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 244-248.

Lauer,J., Shen,C.-K.J., and Maniatis,T. (1980) *Cell*, **20**, 119-130.

Lawn,R.M., Fritsch,E.F., Parker,R.C., Blake,G., and Maniatis,T. (1978) *Cell*, **15**, 1157-1174.

Lee,L.S., and Cheng,Y.C. (1976) *J. Biol. Chem.*, **251**, 2600-2604.

Liebhaber,S.A., Goossens,M., and Kan,Y.W. (1981) *Nature*, **290**, 26-29.

Mantei,N., Boll,W., and Weissmann,C. (1979) *Nature*, **281**, 40-46.

Norgard,M.V., Keen,K., and Monohan,J.J. (1978) *Gene*, **3**, 279-292.

Perucho,M., Hanahan,D., and Wigler,M. (1980) *Cell*, **22**, 309-317.

Proudfoot,N.J., and Baralle,F.E. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 5435-5439.

Rigby,P.W.J., Dieckmann,M., Rhodes,C., and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237-251.

Rutherford,T., Clegg,J.B., Higgs,D.R., Jones,R.W., Thompson,J., and Weatherall (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 348-352.

Southern,E.M. (1975) *J. Mol. Biol.*, **98**, 503-517.

Spandidos,D.A., Harrison,P.R., and Paul,J. (1981) *Biosci. Rep.*, **1**, 911-920.

Stow,W.D., and Wilkie,N.M. (1976) *J. Gen. Virol.*, **33**, 447-458.

Twigg,A.J., and Sherratt,D. (1980) *Nature*, **283**, 216-218.

Ullrich,A., Shine,J., Chirgwin,J., Pictet,R., Tischer,R., Rutter,W.J., and Goodwin,H.M. (1977) *Science (Wash.)*, **196**, 1313-1319.

Wahl,G.M., Stern,M., and Stark,G.R. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 3683-3687.

Wigler,M., Silverstein,S., Lee,L.-S., Pellicer,A., Cheng,Y.C., and Axel,R. (1977) *Cell*, **11**, 223-232.

Wigler,M., Pellicer,A., Silverstein,S., and Axel,R. (1978) *Cell*, **14**, 725-731.

Wilkie,N.M., Clements,J.B., Boll,W., Mantei,N., Lonsdale,D., and Weissmann,C. (1979) *Nucleic Acids Res.*, **7**, 859-877.

Wold,B., Wigler,M., Lacy,E., Maniatis,T., Silverstein,S., and Axel,R. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 5684-5688.