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# Cloning of a new mouse foetal β-globin mRNA sequence

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## ABSTRACT

A novel globin cDNA recombinant (pFG5) has been isolated from a 14-15 day porton mouse foetal liver cDNA library. It codes for a β-like globin mRNA expressed in foetal liver-derived erythroblasts and erythrocytes but not in adult reticulocytes nor in yolk sac derived nucleated erythrocytes. It is also found in Friend cells induced to differentiate by DMSO.

The nucleotide sequence of pFG5 confirms that it does not code for the β major or β minor globin chains nor the embryonic $\epsilon Y_2$ globin chain; but it is identical to the published partial sequence of the $\epsilon Y_3$ globin gene over the region of overlap (78 nucleotides).

## INTRODUCTION

Several globin genes are expressed at different stages of mouse embryonic and foetal development, culminating in the definitive adult α and β globin chains in late foetal development and after birth (for reviews see (1) and (2 )). Three different embryonic haemoglobins are synthesised early in development in nucleated erythrocytes derived from the blood islands of the yolk sac. These are composed of two β-like chains, Z and ε y, in combination with the adult α chain and an α-like embryonic X chain. These haemoglobins are EI ($X_2\epsilon y_2$), EII ($\alpha_2 y_2$) and EIII ($\alpha_2 Z_2$). After the tenth to eleventh day of gestation, the site of erythropoiesis is switched to the foetal liver (1), resulting in the production of enucleated erythrocytes synthesizing the adult α globin chain and one or two adult β globin chains depending upon the strain of inbred mouse (3). Mice designated $Hbb^s$ (single) at the β globin locus make only one adult β globin chain, whereas the $Hbb^d$ (or $Hbb^p$) genotypes result in the synthesis of both the β-major and $\beta^d$-minor or $\beta^p$ minor chains respectively (4, 5, 6). The expression of the y globin genes is related to the genotype at the Hbb locus; $\epsilon y_1$ always

being expressed in association with Hbbs and $\epsilon y_2$ with $Hbb^d$ (7,8). It is, as yet, unclear what pattern of expression is displayed by the recently discovered $\epsilon y_3$ gene.

Unlike the situation in some species, including humans, the existence of a specific foetal $\beta$-like globin in the mouse is controversial: previous evidence supports the contention that the embryonic globin chains are replaced directly by the definitive adult chain. But more recent evidence for a specific foetal $\beta$-like globin chain has been reported (9,10,11) although this is disputed by other workers (12).

In this paper, we report the existence of a $\epsilon Y_3$-like globin mRNA sequence coding for a $\beta$-like globin chain which appears to be uniquely expressed in foetal erythropoietic cells but not in nucleated erythrocytes of yolk sac origin nor in mature adult enucleated erythrocytes. Furthermore, this sequence is expressed in differentiating Friend cells in addition to the expression of the $\beta$ major and $\beta$ minor chains.

## MATERIALS AND METHODS

### Normal erythroblasts and erythrocytes:

Foetal livers were dissected from 13-15d Porton Swiss mouse foetuses and peripheral blood from 12-15d foetuses was obtained by bleeding from the umbilical cord. Foetal liver-derived enucleated red blood cells were separated from nucleated cells (including yolk-sac derived nucleated erythrocytes) by elutriation on the Beckman JE-6B rotor. The enucleated cells were elutriated at $4^o$ at a flow-rate of 11 ml/min with centrifuge speed of 2000 r.p.m. Yolk-sac derived nucleated erythrocytes in foetal peripheral blood (plus any other large nucleated white cells present) were separated from enucleated foetal liver-derived erythrocytes by collecting cells elutriating at $4^o$ at 2000 r.p.m. between flow rates of 12-30 ml/min. Successful cell fractionation was monitored using stained preparations of elutriated cells.

### Friend Cells

Friend cells (clone M2) seeded at $5x10^4$/ml were grown for 5d in the absence or presence of 1.5% ($^v/_v$) dimethyl-sulphoxide to a density of $10^6$/ml in Eagles Medium containing 10% foetal calf serum.

### Preparation of Cytoplasmic RNA

Total cytoplasmic RNA was prepared by precipitation with lithium chloride/urea as described previously (13, 14). Polyadenylated mRNA was

selected on T-3 oligo d(T)-cellulose (Collaborative Research) as described by Affara et. al. (15).

Preparation of Single-Stranded cDNA

Single-stranded cDNA (labelled with $^{32}$P-dCTP or $^{3}$H-dCTP) was prepared as described by Affara et. al. (15). $^{3}$H-labelled cDNA had a specific activity of $10^{7}$cpm/$\mu$g and $^{32}$P-labelled cDNA of $2\times10^{8}$cpm/$\mu$g. The reverse transcriptase was provided by courtesy of Dr. J. W. Beard, Life Science Inc., USA.

Synthesis of Double Stranded cDNA

Using cytoplasmic poly$A^{+}$ mRNA from 14-15 day mouse foetal liver as template, cDNA (labelled to a specific activity of $2\times10^{5}$ cpm/$\mu$g with $^{3}$H-cdCTP) was prepared as described above except that the actinomycin D was omitted from the reaction. After the incubation, the reaction mixture was made up to 0.5% SDS, 0.1M EDTA and 0.2M NaCl, extracted twice with CHCl3/iso-amyl alcohol (24:1) (CIA), made to 0.4M NaOH and incubated at 37$^{o}$ for 3 h. After neutralization ,the cDNA was vacuum-concentrated against DNA polymerase buffer (30mM Tris-HCl, pH7.5, 4mM $MgCl_2$). The second strand of cDNA was extended using dGTP, dATP, dCTP and dTTP (1mM each), 10mM by 2-mercaptoethanol and 200 units/ml DNA polymerase (Klenow fragment, Boehringer Corporation). After incubation at 25$^{o}$C for 5 h, the reaction was extracted twice with SDS/NaCl/CIA and then extensively vacuum dialysed against 90mM sodium acetate, 150mM NaCl, 3mM $ZnSO_4$ and 15% glycerol. The dialysate was diluted three times, 30 units/ml of S1 nuclease (BRL) added, for 2 h at 37$^{o}$ and then extracted twice with SDS/NaCl/CIA. The reaction was dialysed against DNA polymerase buffer, incubated for 2h with DNA polymerase (under the same conditions as described above), and extracted twice with SDS/NaCl/CIA before dialysis against ligase buffer (30mM Tris pH9.0, 4mM $MgCl_2$, 10mM dithiothreitol, 1.2mM EDTA). The mean size of the double-stranded cDNA (as determined on alkaline/sucrose gradients) was 350 base-pairs.

Preparation of Plasmid DNA for Blunt-End Ligation

The plasmid pAT153 was propagated in E.Coli HB101 grown in L-broth containing 100$\mu$g/ml of ampicillin. Superhelical plasmid DNA was purified by equilibrium banding in CsCl followed by sedimentation of the DNA through neutral 5-20% sucrose gradients (16). 50 $\mu$g of superhelical plasmid were completely linearized by restriction with Hind III, extracted twice with CIA, alcohol precipitated and the plasmid DNA taken up in DNA polymerase buffer and the extremities repaired using DNA

polymerase (as described above). After CIA extraction and alcohol precipitation, the plasmid DNA was taken up in 500 µl of 50 mM tris pH8.0 and treated with 2.5 units of calf intestine alkaline phosphatase at 37°C for 30 min, extracted twice with CIA, alcohol precipitated and finally taken up in 10 mM NaCl, 10 mM tris pH7.5 1 mM EDTA.

Blunt-End Ligation

Equimolar amounts of phosphatase-treated plasmid and double-stranded cDNA were taken up in 20 µl of ligation buffer containing 30 mM Tris pH8.0, 4 mM $MgCl_2$, 10 mM dithiothreitol, 1.2 mM EDTA, 50 µg/ml Bovine Serum Albumin (RIA grade, Sigma), 1 mM ATP and 1.2 units of T4 DNA ligase (BRL). The reaction was incubated for 18 h at 12°, a further 1.2 units of DNA ligase added, and incubation continued for a further 18 h.

Transformation of Bacteria

The ligation mixture was used directly to transform E.Coli HB101 as described by Fantoni et. al. (17) and the transformants selected on soft agar medium containing 100 µg/ml ampicillin.

Colony Hybridisation

Recombinant colonies were streaked on to master plates and then inocculated into 96-well microtitre plates containing L-broth with ampicillin at 100 µg/ml. Replicas of the colonies in the microtitre plates were made on nitrocellulose filters (Millipore) using a transfer plate, grown overnight and prepared for colony hybridization (Grunstein and Hogness, 18) using 2-10x$10^6$ cpm $^{32}P$ labelled cDNA.

Nick Translation

Labelling of plasmid DNA with ($\alpha$ -$^{32}P$) dCTP was performed using the Amersham Nick Translation Kit (N5000). Specific activities of 1-3x$10^8$ cpm/µg were routinely obtained. Where a plasmid DNA gave low levels of incorporation, further purification of the DNA using methoxy-ethanol phosphate (19) resulted in improved specific activity after nick translation.

Restriction Enzyme Digestions

2 µg of purified plasmid DNA was digested to completion in a 10 µl reaction volume containing 50 mM NaCl, 40 mM Tris/HCl pH7.4, 60 mM $\beta$-mercaptoethanol and 10 mM $MgCl_2$. Restriction digests were then analysed by electrophoresis in 6% acrylamide, 0.16% bisacrylamide gels made up in 40 mM Tris pH8.3, 20 mM sodium acetate and 2 mM EDTA.

Hybridization Selection of mRNAs for In Vitro Translation

(a) Binding of Recombinant DNA to Activated DBM Paper: Recombinant DNA,

restricted with endonuclease Hinf 1 was bound to activated 1cm diam. discs of DMB paper as described previously (21).

(b) Hybridisation of mRNA: Discs with bound plasmid DNA were prehybridized in microtitre plates for 5 h at 42°C in 200 µl of 50% deionized formamide, 0.75M NaCl, 0.1M Tris/HCl pH7.5, 1mM EDTA, 0.5% SDS, 200 µg/ml wheat germ tRNA and 100 µg/ml poly rA. 10 µg of poly $A^+$ mRNA was taken up in 140 µl of the same buffer and incubated with the disc at 42°C for 20 h with rotary agitation.

(c) Elution of Hybridized mRNA: After hybridization, the discs were given two 5 min washes at 20° in 1xSSC, 0.5% SDS, 2mM EDTA, three 5 min washes in the same buffer at 60°C, three 5 min washes in 0.1 x SSC, 0.1% SDS, 2mM EDTA at 60°C and finally two 5 min washes in 10mM Tris pH7.5, 2mM EDTA at 60°C. mRNA was then eluted by two 15 min washes at 42°C with 140 µl 95% formamide, 10mM Tris pH7.5, 2mM EDTA, with constant rotary agitation. The eluates were pooled, 2 µg of wheat germ tRNA added, sodium acetate added to 0.3M and the mRNA precipitated with 5 vol ethanol at -80°C.

In Vitro Translation

The pelleted RNA was washed in 70% ethanol at 4°C, dried under vacuum and dissolved in sterile water for translation at 25° for 75 min. in 18 µl volume reactions using the BRL wheatgerm *in vitro* translation kit. The synthesized polypeptides were analysed on polyacrylamide acid/urea gels containing 15% acrylamide, 0.9% bis-acrylamide, 6.25M urea (Schwartz-Mann ultra pure), 5.4% ($^{v}/_{v}$) acetic acid, 5% TEMED and 0.125% ammonium persulphate. The samples were applied in a buffer containing 4M urea, 1.4M β-mercaptoethanol, 5% ($^{v}/_{v}$) acetic acid and 15% ($^{w}/_{v}$) sucrose and electrophoresed towards the cathode in 0.9M acetic acid at 14 m.a. for 5 h. The gel was fixed in 10% trichloroacetic acid, 10% acetic acid, 30% methanol and 2.5% glycerol, then treated for fluorography by soaking in Enhance solution (New England Nuclear) for 1 h, followed by two 15 min. washes in distilled water and finally dried on to Whatman 3MM paper

Determination of mRNA Concentration by Filter Hybridization to Cloned cDNA recombinants

Hinf1-restricted plasmid DNA was denatured for 30 min. in 0.8ml/0.5M NaOH, 0.5xSSC at 20°, neutralized and then filtered slowly under vacuum on to 1.2cm Millipore nitrocellulose discs which had previously been washed with 5ml of 6xSSC. After filtering the DNA, the disc was washed with a further 5ml of 6xSSC, followed by a wash with a 5ml of 6xSSC, then

rinsed gently in 70% ethanol and baked at 80°C for 3-4 h. Before hybridization with cDNA (labelled to a specific activity of $5x10^6$ cpm/µg, filters were prehybridized for 16-20 h at 42°C in 1 ml of 5xSSC, 5x Denhardts solution, 50% ($^v/_v$) formamide and 200µg/ml salmon sperm DNA. Filters were then hybridized with increasing amounts of cDNA ($5x10^4$ to $3x10^5$ cpm) for 16-20 hours at 42°C in 200µl 5xSSC, 1xDenhardts solution, 50% ($^v/_v$) formamide and 200µg/ml of salmon sperm DNA in microtitre plates with gentle rotary agitation. The filters were subsequently washed twice at 20° for 5 min. in 0.1xSSC, 0.1% SDS, followed by three 20 min. washes in the same buffer at 60°C.

DNA Sequencing

All procedures for DNA sequencing were performed as described by Maxam and Gilbert (22). DNA fragments for sequencing were purified by electrophoresis in a 1% agarose gel (after appropriate restriction digestion) and the desired fragment extracted from the gel by electro-elution into a sealed dialysis sac. DNA was subsequently recovered by alcohol precipitation and used for the DNA cleavage reactions to permit sequencing.

## RESULTS

### Isolation of globin cDNA recombinant pFG5.

Cytoplasmic poly $A^+$ mRNA from 13-15 d mouse foetal livers was used to prepare double-stranded cDNA for cloning into plasmid pAT153 in order to establish a cDNA library of erythroid cell mRNAs. The ds cDNA molecules were introduced into the HIND III site of the vector by blunt-end ligation using T4 DNA ligase.

Recombinant colonies were picked and grown up in microtitre plates and then replica-plated on to nitrocellulose filters, grown and lysed using the Grunstein and Hogness protocol (18). Nitrocellulose-bound recombinants were subsequently screened either with $^{32}$P-labelled cDNA from foetal liver poly $A^+$ mRNA or foetal liver cDNA previously hybridized in solution with 100µg each of HhaI-restricted pCR1 plasmid recombinants containing cDNAs coding for mouse $\alpha$ and $\beta$ globins (gifts from Dr. C. Weissman, Zurich). By comparison of the two filter hybridizations, recombinants containing cDNAs coding for adult $\alpha$ and $\beta$ globin sequences were eliminated (results not shown). Colonies showing weak or no competition with the adult globin cDNA recombinants were selected for further characterization. One such cDNA recombinant (pFG5), proved to

contain an insert coding for a foetal globin mRNA.

## The Sequence of the pFG5 cDNA Insert

Proof that the pFG5 sequence codes for a globin mRNA specifying a β-like globin chain was obtained by DNA sequencing studies.

The DNA sequencing strategy employed is illustrated by the restriction map of the insert shown in figure 1. The following pFG5 digests were performed and the fragments analysed on 6% polyacrylamide gels: ClaI, ClaI plus BamH1, BamHl, EcoRl, EcoRl plus BamHl and EcoRl plus Hinfl. By these criteria, pFG5 contains sites for BamH1 and EcoR1 but not for ClaI. The positions of these sites within the insert have been mapped with reference to known sites in the plasmid surrounding the point of insertion at the Hind 111 site (i.e. the BamH1, ClaI, and EcoR1 sites).

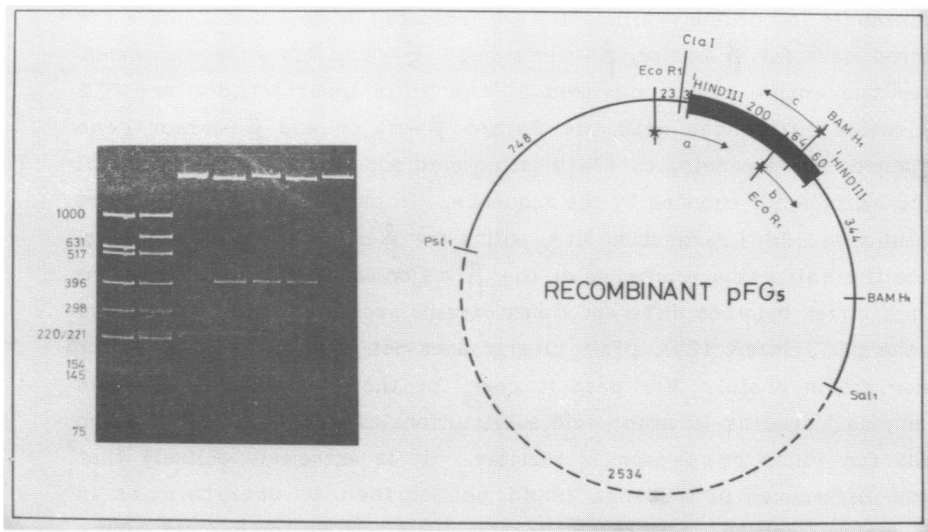


Figure 1. Restriction Analysis of pFG5.

2 μg pFG5 DNA was restricted with various enzymes and the fragments analysed on a 6% polyacrylamide gel. The digests from left to right are: pAT restricted with Hinf1 and EcoR1; pFG5 with Hinf1 and EcoR1, EcoR1 alone, BamH1 and EcoR1, BamH1 alone, ClaI and BamH1 and finally ClaI alone. The restriction map shows the sequencing strategy used. The end labelled sites are marked with a star and the direction of sequencing is indicated by an arrow. A partial EcoRI digest was used to sequence from the pAT153 EcoRI site across the EcoRI site within the insert. The lengths of sequence obtained from the three reactions were: a, 188 nt; b, 111 nt; c, 107 nt.

The following restriction fragments were prepared and end labelled with $^{32}P$ (using T4 DNA polymerase). a) A partial digestion fragment from the pAT153 EcoR1 site to the pAT153 Pst1 site, labelled at the EcoR1 site; (b) a fragment extending from the insert EcoR1 site to pAT153 Pst1 site, labelled at the EcoR1 site; (c) a fragment from the pAT153 Pst1 site to the insert BamH1 site, labelled at the BamH1 site. The arrows in figure 1 show the direction of sequencing from the sites of labelling. Thus the entire pFG5 sequence was obtained including two stretches of the sequence (31 and 46 nucleotides) confirmed by sequencing in both strands.

Figure 2 shows the entire sequence of pFG5 (including the encoded amino acids) with those of adult Balb/c β-major and β-minor globin mRNAs (23), and the available amino acid sequence of the $\epsilon Y_2$ globin chain (6). Also shown is the available nucleotide sequence of a recently discovered Balb/c β-globin gene designated $\epsilon$ y3 (24) together with the amino acid sequence of the putative globin chain encoded. Differences in homology with pFG5 are indicated by an asterisk above the altered base for β-major, β-minor and $\epsilon$ y3 globin gene sequences. Over the entire region covered by the pFG5 insert, there are 27% nucleotide differences with the Balb/c β-major and β-minor gene sequences corresponding to 17-19 amino acid substitutions out of a total of 68 amino acids encoded by the sequence. In particular the pFG5 insert contains an ECoR1 restriction site, unlike the β major and β minor genes. Since the amino acid sequences of the β major or β minor globin chains do not differ between different mouse strains over the region represented by the pFG5 insert (29), pFG5 clearly does not code for a β major or β minor globin chain. Nor does it code for the embryonic $\epsilon y_2$ globin chain, as judged by 10 amino acid substitutions over a region of 44 amino acids for which comparison is possible. It is extremely unlikely that these differences in sequence could be ascribed to uncertainties in sequencing since 16/90 of the nucleotide substitutions (or 5-7 out of the 10-19 corresponding amino acid substitutions encoded) occur in the two regions of the pFG5 insert for which the sequence has been confirmed by sequencing both strands.

However, over the available sequence overlap with the $\epsilon y_3$ coding sequence (78 nucleotides) there is complete homology with pFG5, indicating that they are probably the same sequence.

Tissue specificity of expression of the pFG5 globin mRNA.

To establish the cell specificity of expression of the globin mRNA

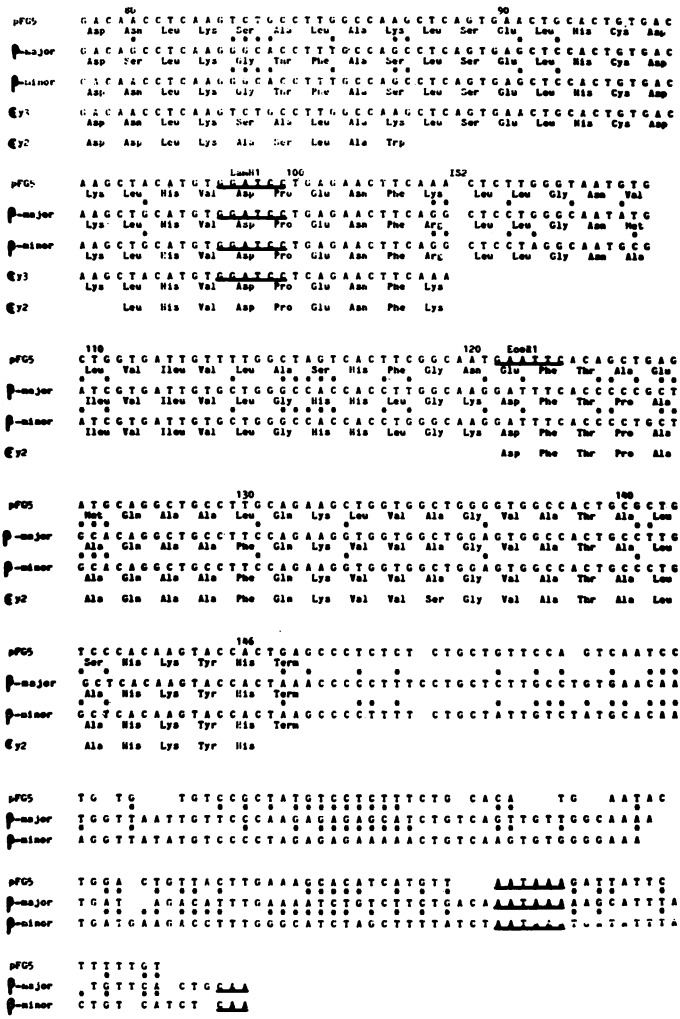

Figure 2. The pFG5 insert nucleotide and corresponding amino acid sequences are displayed aligned against the published sequences for β-major, β-minor and ε $y^3$ (24), this latter gene being a recently discovered putative embryonic β-globin sequence (see text for details). Differences between pFG5 and the other gene sequences have been indicated by an asterisk above the appropriate nucleotide. Sequences beyond the termination codon have not been kept in strict frame but shifted to maximise the homology. This indicates that deletions and insertions in the 3' non-coding region have occurred between the different β globin genes. The common BamH1 site and the EcoR1 site in pFG5 have been underscored as has the putative polyA addition signal sequence (AATAAA) and the putative polyA addition site (CAA) at the 3' end. IS2 represents the second intervening sequence. The availab le amino acid sequence of the ε $Y_2$ globin chain (6, 29) is also included.

encoded by pFG5 in erythroid cells from different stages of mouse development, hybridization-selection of pFG5 mRNA by pFG5 plasmid DNA bound to DBM paper discs was performed using mRNA from embryonic and foetal erythrocytes, adult reticulocytes and induced Friend cells. The selected mRNA was subsequently translated in the wheat germ system and the synthesized polypeptides analysed on acid/urea gels (Figure 3). The acid/urea gels clearly resolve the adult $\alpha$ and $\beta$ globin chains; the embryonic $\beta$ -like globin chains appear to be represented by the intense slowly migrating band (lane j).

The results (lanes e and f) show that adult $\alpha$ and $\beta$ recombinant cDNAs specifically select their respective mRNAs from induced Friend cell mRNA

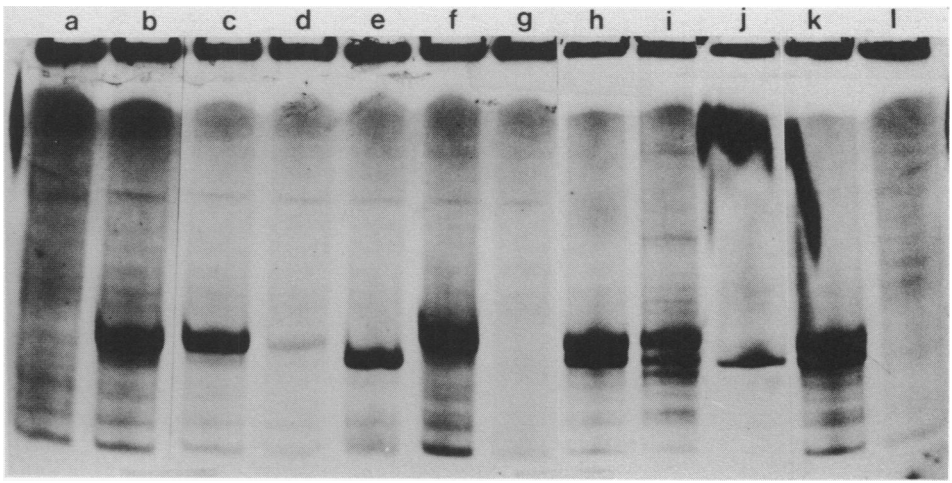


Figure 3. Hybridization Selection of mRNA.

10$\mu$g of poly$A^+$ mRNA prepared from erythroid cells at different stages of mouse development was hybridized with 20$\mu$g cDNA recombinant DNA which had been restricted and bound to DBM paper discs as described in the materials and methods. After extensive washing, hybridized mRNA was eluted from the discs and translated in a wheat germ in vitro translation system. The protein products were then analysed on an acid/urea gel. Lanes a-d show, respectively, the translation of mRNA selected by pFG5 from embryonic nucleated erythrocytes, foetal enucleated erythrocytes, induced Friend cells and adult reticulocytes. Lanes e and f show the translation of mRNA selected from induced Friend cells by adult $\alpha$ and $\beta$ globin cDNA recombinants. Lane g shows the pAT control with induced Friend cell mRNA and lanes h-k show, respectively, the translation of adult reticulocyte mRNA, induced Friend cell mRNA, nucleated erythocyte mRNA and foetal enucleated erythrocyte mRNA Lane L is a no message control.

(and also from adult reticulocyte and foetal erythrocyte mRNA - results not shown). By contrast, under the conditions of high stringency employed, pFG5 recombinant DNA strongly selects a mRNA coding for a β-like chain only from induced Friend cells and foetal erythrocytes (lanes b and c). This result indicates that pFG5 mRNA is not found in embryonic or adult erythroid cells but that it is specifically expressed in foetal red blood cells. In addition it is interesting to note that Friend cells, like foetal erythrocytes, express both the adult globin chains and the pFG5 β-like chain.

The level of pFG5 mRNA sequences in the cytoplasmic poly $A^+$ mRNA of a number of cell types was quantitated by measuring the concentration of its complementary sequences in cDNAs transcribed from the various mRNA populations. Increasing amounts of each cDNA were titrated against an excess amount of restricted and denatured pFG5 (or, as controls, α or β globin cDNA) recombinant DNA bound to nitrocellulose discs, so that the percentage of cDNA bound to the filter was proportional to the concentration of the appropriate mRNA sequence in the population, assuming equal efficiency of transcription into cDNA of all the mRNAs in a mixed population. Figure 4 illustrates results for poly $A^+$ mRNAs obtained from foetal liver cells, adult mouse reticulocytes, mouse primary fibroblasts, uninduced Friend erythroleukaemia cells and erythroleukemia cells induced to undergo differentiation by treatment with DMSO for 6 d. Since the concentrations of α and β globin mRNAs in Friend cells and reticulocytes are accurately known from liquid hybridisation experiments, these control filter hybridisation experiments with α and β globin cDNA recombinants serve to show the validity of the filter hybridisation method for these purposes. It is evident from figure 4 that pFG5 mRNA is present at much lower concentrations than adult globin mRNAs in the mRNA populations of foetal liver cells, uninduced Friend cells and adult mouse reticulocytes. It is not detectable in primary mouse fibroblast mRNA, (limit of detection $< 0.1\%$). However, upon treatment of Friend cells with DMSO for six days the level of pFG5 mRNA increases to a similar level to adult α and β globin mRNAs (Table 1).

## DISCUSSION

### pFG5 mRNA is Expressed Specifically During Foetal Development

Incontrovertible evidence for the existence of globin chains

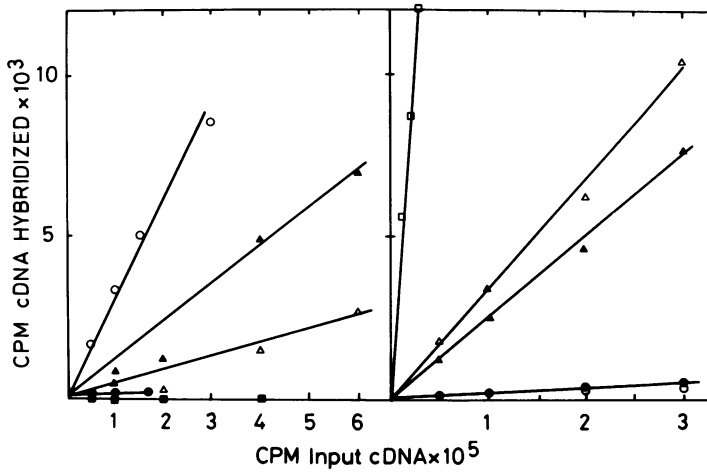


Figure 4. Determination of pFG5 mRNA Levels.

The level of pFG5 mRNA sequences in different erythroid cells was measured by hybridizing increasing quantities of $^{3}$H-labelled cDNA with recombinant DNA bound to nitrocellulose filters. The cDNA cpm bound to the filter is plotted against the total cpm applied. The slope of the line obtained is proportional to the concentration of the sequence in the population. The left-hand panel shows the hybridization of pFG5 DNA with induced Friend cell cDNA (O–O), uninduced Friend cell cDNA (● -●), foetal liver cDNA ( △ - △ ), and primary fibroblast cDNA ( ■ - ■ ). The right-hand panel shows the hybridization of uninduced Friend cell cDNA with α globin cDNA recombinant DNA ( ● - ● ) or β globin cDNA recombinant DNA (O–O); induced Friend cell cDNA with α (▲-▲ ) or β (△ - △ ) globin cDNA recombinant DNAs; reticulocyte cDNA with β -globin cDNA recombinant DNA ( □ - □ ).

expressed specifically during the foetal phase of mouse development has proved elusive. The putative foetal globin chains may electrophorese or chromatograph in a similar fashion to the adult chains (as we have found for pFG5 and β -major) and hence cannot be distinguished. Alternatively, differently migrating haemoglobin and globin bands could arise by modification of adult globin chains. Whitney et al. (12) have explained the putative foetal haemoglobin reported by Kraus et al. (9) on this basis. In addition, Whitney (25) has proposed that the decrease in β -minor globin chains and increase in β -major chain synthesis in early postnatal life of the diffuse strains of mice is the murine counterpart of foetal to adult haemoglobin switching in other mammals. Nevertheless, Wu et al. (10,11) have reported a mouse foetal haemoglobin whose globin chains are indistinguishable from adult α globin on acid/urea gels, but

TABLE 1

Globin mRNA levels in mouse erythroid cells (% total mRNA)

| Recombinant DNA | Reticulocyte mRNA | Foetal Liver mRNA | Friend Cell mRNA | Friend Cell mRNA + DMSO |
|---|---|---|---|---|
| pCR1α | 36 | 13 | 0.17 | 2.72 |
| pCR1β | 36 | 5 | 0.17 | 3.6 |
| pFG5 | 1.25 | 0.46 | 0.14 | 3.2 |

Table 1. Summary of pFG5 mRNA Levels.

At completion, filter bound adult β globin cDNA recombinant DNA could hybridize 36% of input reticulocyte cDNA a figure in good agreement with liquid hybridization titrations. The slope of this titration was taken as a standard to which the other titrations were compared. The results shown are based on experiments performed in a similar fashion to those shown in figure 4.

which differ by immunological criteria.

Such interpretational problems do not arise in regard to the present studies, since we have defined the status of the pFG5 gene at the nucleotide level. Also using the translational assay of mRNAs selected from different erythroid tissues by hybridization with pFG5 DNA, we have shown that a strong β-like globin chain can only be obtained by translating mRNA selected from purified enucleated erythrocytes of foetal liver origin or induced Friend cells, but not from purified nucleated erythrocytes of yolk sac origin or adult reticulocytes. The faint band shown in the translational assay with reticulocyte polyA$^+$ mRNA probably reflects weak cross-hybridization with adult β globin mRNA.

The induction of the pFG5 foetal globin chain in differentiating Friend cells corroborates the report of Wu and Zucker (11) in which evidence is presented for induction of a foetal haemoglobin in clone T3C12 Friend cells. The presence of the pFG5 sequence in our Friend cells (clone M2 derived originally from clone 745 cells) suggests that Friend cell differentiation is characteristic of foetal rather than adult red cell maturation.

## The Mouse Globin Gene Cluster

It has recently been shown that the organization of the β globin gene cluster in the mouse (24) is similar to humans (26) and rabbits (27, 28). In the Balb/c mouse Jahn et al. (24) have mapped the following globin gene order: 5'-εy3-βho-βh1-βh2-βh3-β-major-β-minor-3'. From their amino acid and nucleotide sequences, the βho, βh1 and βh2 genes would appear to be new β-like genes, whereas the βh3 sequence displays the attributes of a pseudogene. The εy3 gene has been designated as a third embryonic gene by Jahn et al. (24) because of its close homology in amino acid sequence to εy2 (see Gilman (6) and Steinheider et al. (28)), and its strong hybridization to a cDNA clone isolated by Fantoni et al. (17) from mRNA of nucleated mouse erythrocytes. However, over the entire region of overlap between the cloned pFG5 and εy3 sequences (amino acids 79-146 in figure 2) there is absolute homology, strongly suggesting that they are the same gene, (of course, differences may exist in other areas of their respective sequences) and functionally, we have designated pFG5 as a foetal gene on the basis of its location in purified foetal liver derived erythroid cells separated by elutriation. If indeed pFG5 and εy3 are the same gene in their entirety, the inability of pFG5 to hybridize to mRNA from nucleated erythrocytes can be resolved by the fact that much higher stringency criteria were used in these studies for the hybridization selection of mRNA. The conditions used by Jahn et al. would tolerate a greater degree of base mis-match.

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