Proc. Nat. Acad. Sci. USA
Vol. 72, No. 1, pp. 258–262, January 1975

Human Globin Gene Expression and Linkage in Bone Marrow and Fetal Liver

(complementary DNA/fetal globin mRNA/translational control)

W. GEORGE LANYON, SERGIO OTTOLENGHI, AND ROBERT WILLIAMSON

The Beatson Institute for Cancer Research, 132 Hill Street, Glasgow G3 6UD, Scotland

Communicated by Donald D. Brown, October 2, 1974

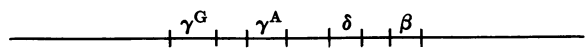
ABSTRACT **During embryonic development there is a transition from embryonic and fetal to adult β-type globin chains. The high-molecular-weight RNA found in nuclei from embryonic and adult human erythropoietic tissues, fetal liver, and bone marrow, have been investigated for the presence of γ(fetal)- and β(adult)-globin messenger RNA sequences by molecular hybridization. Unlike α- and β-globin mRNA sequences, γ-globin mRNA sequences are absent from both total and high-molecular-weight nuclear RNA isolated from adult bone marrow. The amount of cytoplasmic γ-globin mRNA is proportional to the level of γ-chain synthesis, demonstrating that translational control is not a major control mechanism in the expression of globin genes. Since the γ-, δ-, and β-globin genes are known to be closely linked genetically, transcriptional control can discriminate between similar gene sequences that are spatially adjacent to one another.**

During embryonic development cells in the fetal liver are mainly erythropoietic, while in adults erythropoiesis is normally confined to a smaller proportion of nucleated cells in bone marrow. These fetal liver and adult marrow erythroid cells are nucleated, and make many other proteins besides hemoglobin. In both fetal and adult blood, immature non-nucleated circulating reticulocytes are found in low numbers, which synthesize hemoglobin almost exclusively, and are thus a source of pure globin messenger RNA (mRNA). This pure globin mRNA can be transcribed at high specific radioactivity with viral reverse transcriptase to yield complementary DNA (cDNA) (1–10).

By use of such a cDNA probe, globin mRNA sequences can be measured even when present in very small amounts. Such a technique has been used to demonstrate the presence of globin mRNA sequences in high-molecular-weight nuclear RNA (HnRNA) isolated from duck erythroblasts (11). Globin mRNA sequences have also been demonstrated in HnRNA by sensitive translation assays (12–14).

Several models of the relationship between mRNA and HnRNA have been postulated, and are summarized in a recent review by Darnell *et al.* (15). It has been shown that there is a poly(A) sequence at the 3′-terminus of many HnRNA molecules, that this is conserved during processing to the cytoplasm, and that sequences with the characteristics of mRNA are adjacent to this poly(A) tract at the 3′-end. Therefore, the simplest model of the relationship between HnRNA and mRNA is a long primary transcript with a single mRNA terminating in a poly(A) sequence, which is processed to the cytoplasm, while the remaining sequence at the 5′-end of the HnRNA molecule is broken down in the nucleus. An alternative model predicts that there are several mRNA sequences towards the 5′-end of the HnRNA molecule, but only the mRNA at the 3′-end is processed, perhaps by virtue of its poly(A) sequence (16).

The genes specifying the β-type globin chains provide a system in which it is possible to examine nuclear RNA for the presence of mRNA sequences that might be transcribed but are not expressed at the translational level. The developmental sequence of globin appearance is mirrored in the arrangement of the genes on the chromosome (17, 18):



The γ-, δ-, and β-chain genes are closely linked, as shown by the occurrence of β-type globins in which the NH_2-terminal sequence of γ- or δ-globin is linked to the COOH-terminal sequence of β-globin by a mutation at the gene level (17, 18). It has been widely suggested that this indicates the absence of other structural genes from the regions between the γ-, δ-, and β-globin genes (17, 18).

By preparing purified γ-globin cDNA from transcripts of fetal reticulocyte globin mRNA, it has been possible to demonstrate the absence of significant amounts of γ-globin mRNA in nuclear RNA from human bone marrow, which contains a high level of transcripts of β-globin mRNA.

MATERIALS AND METHODS

Isolation of Human Tissue. Adult human reticulocytes were obtained from patients with nonthalassemic hemolytic anemia and an absence of demonstrable hemoglobin F; the reticulocyte counts varied up to 40%. Adult human bone marrow was obtained from normal ribs removed during thoractomy of patients with no hematological disorder. Fetal livers were obtained from 10 to 18-week fetuses after hysterotomy; in no case were there pathological abnormalities. Neonatal reticulocytes were obtained from cord exchange transfusion blood from infants with Rhesus incompatibility.

Isolation of mRNA. Messenger RNA was prepared by affinity chromatography with oligo(dT)–cellulose (Collaborative Research, Waltham, Mass.) from total polysomal RNA (for reticulocytes) and from total RNA prepared from the supernate after centrifugation of a cell homogenate at 2000 × *g* for 10 min (for fetal liver or bone marrow) (19). The RNA was extracted with phenol–chloroform (20). Approximately 2% of the total RNA was retained by the oligo(dT)–cellulose column. The purity of each mRNA preparation was estimated by polyacrylamide gel electrophoresis.

Assay for mRNA Translational Activity. Messenger RNA was assayed for translational activity in a wheat germ cell-free system containing [^{14}C]leucine (21). After incubation for 90 min, 30 mg of carrier human hemoglobin (prepared

Abbreviations: cDNA, complementary DNA prepared, with reverse transcriptase of avian myeloblastosis virus, from messenger RNA; HnRNA, high-molecular-weight nuclear RNA; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

from neonatal exchange transfusion blood) was added, and the globin was extracted and chromatographed on carboxymethylcellulose (22).

Isolation of Nuclear RNA. Nuclei were prepared by the citric acid procedure outlined by Busch and Smetana (23). Nuclei were suspended in ANE buffer [10 mM sodium acetate, 0.1 M NaCl, 1 mM EDTA, 0.5% sodium dodecyl sulfate (pH 6.0)] and extracted with phenol–chloroform until no interface was visible. After ethanol precipitation, the total nucleic acids were dissolved in HMC buffer [10 mM magnesium acetate, 25 mM NaCl, 2 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid $CaCl_2$, 0.1 M (Hepes) (pH 7.0)] and incubated for 5 min at 4° with 10 μg/ml of electrophoretically pure DNase (Worthington Corp.). Sodium dodecyl sulfate was added to 0.5% and the preparation was again extracted with phenol–chloroform. The nucleic acids were precipitated with ethanol and then passed through a column of Sephadex G-50 (Pharmacia) to remove DNA fragments.

The excluded peak was precipitated with ethanol (total nuclear RNA) and centrifuged on 15–30% sucrose gradients in NETS buffer [0.1 M NaCl, 10 mM EDTA, 10 mM Tris, 0.2% sodium dodecyl sulfate (pH 7.4)], either in a zonal rotor (12) or for 16 hr at 15,000 rpm in a 3 × 23-ml swing-out rotor (M.S.E.). The small amount of residual 28S RNA served as a size marker, and RNA sedimenting more rapidly than about 30 S was pooled and precipitated with ethanol.

Deionized formamide was prepared with a mixed bed resin [Biorad AG 501x8 (D)] and a formamide buffer was prepared containing 95% formamide, 5% water, 10 mM Hepes, 1.0 mM EDTA, apparent pH 7.5. Sucrose was added to this formamide buffer, and 4–25% (w/w) gradients were prepared. RNA in buffered formamide was layered on the gradients and centrifuged for 18 hr at 55,000 rpm at 20° in a 3 × 6.5-ml swing-out rotor (M.S.E.). Ribosomal RNA and 9S mouse globin mRNA were centrifuged in parallel tubes to provide size markers. The gradients were displaced through an LKB Uvicord (280 nm) with Fluorocarbon 43.

Preparation of Complementary DNA. cDNA was prepared with adult and fetal reticulocyte mRNA as template and oligo$(dT)_{12-18}$ as primer with reverse transcriptase prepared from avian myeloblastosis virus (a gift from Dr. Beard, Duke University, North Carolina) (9), with dCTP (13.3 Ci/mmol, Radiochemical Centre, Amersham) as the only labeled deoxynucleotide. The cDNA was purified on alkaline sucrose gradients, and fractions sedimenting at greater than 5 S were taken, of approximate mean molecular weight 130,000 (9).

Hybridization of cDNA to mRNA. Between 40 and 120 pg of cDNA in 4 μl were incubated in sealed capillaries with various amounts of RNA in a hybridization solution containing 0.5 M NaCl, 25 mM Hepes, 10 mM EDTA (pH 6.8), 50% formamide, 500 μg/ml of *Escherichia coli* RNA for 6–21 days at 43° (9). Some hybridizations were performed at 49°, as indicated in the figure legends, to determine whether mismatched hybrids of low stability were formed at the lower temperature. In every case the time of hybridization was 10–20 times that determined for 50% reannealing of cDNA to homologous sequences, ensuring the hybridization of homologous sequences is essentially complete (10). The percentage of cDNA that had hybridized was determined by assay with single-strand-specific nuclease S1 followed by perchloric acid precipitation of the undigested material (9). Estimation of cDNA complexity was performed by measuring the rate of reannealing of 500 pg of cDNA at a concentration of 25 ng/ml to its template at various cDNA:mRNA ratios under standard hybridization conditions (10).

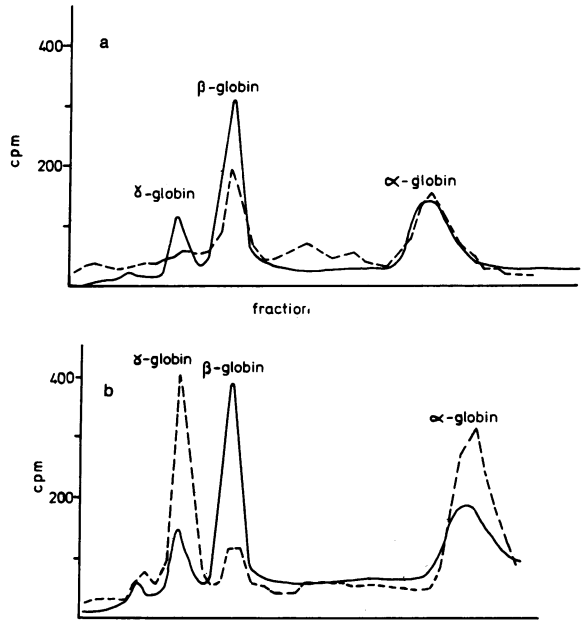


FIG. 1. Incorporation of [^{14}C]leucine into α-, β-, and γ-globin chains in a wheat germ cell-free system. (a) Adult reticulocyte mRNA (1 μg); (b) fetal reticulocyte mRNA (3 μg). Absorbance trace of carrier neonatal hemoglobin (solid line); radioactivity incorporated (dashed line).

Preparation of γ-Enriched cDNA. cDNA (50 ng) from fetal reticulocytes in 80 μl was hybridized to a 5-fold excess of mRNA from adult reticulocytes over that required to obtain the plateau value, as measured by hybridization, and incubated at 49° for 3 days. The hybridization mixture was fractionated into single-stranded (nonhybridized) and double-stranded (hybridized) sequences by successive elution from hydroxylapatite with 0.14 and 0.40 M sodium phosphate (pH 6.8) at 68°. Between 60 and 70% of the cDNA behaved as hybrid. The nonhybridized cDNA was dialyzed three times against 20 volumes of distilled water, incubated for 18 hr at 37° in 0.3 M NaOH to hydrolyze RNA, and neutralized. The cDNA was precipitated in ethanol with 200 μg of *E. coli* carrier RNA. After the cDNA was redissolved in water, it was put through a Sephadex G-25 column above a pad of "Chelex 100" resin equilibrated with distilled water. The excluded peak of cDNA was lyophilized.

Hybridization to Nuclear RNA. The conditions of hybridization to nuclear RNA are identical to those above except that the hybridization mixture was heated to 85° for 10 min to ensure denaturation of the RNA (ref. 11; Harrison, P. R., personal communication).

RESULTS

Translational Activity of Globin mRNAs. The synthesis of globin in wheat germ cell-free systems primed with adult and fetal reticulocyte mRNAs is shown in Fig. 1. The adult reticulocyte mRNA directed the synthesis of 57% α-globin and 43% β-globin; the fetal mRNA, 55% α-globin, 13% β-globin, and 32% γ-globin. In each case more than 80% of the

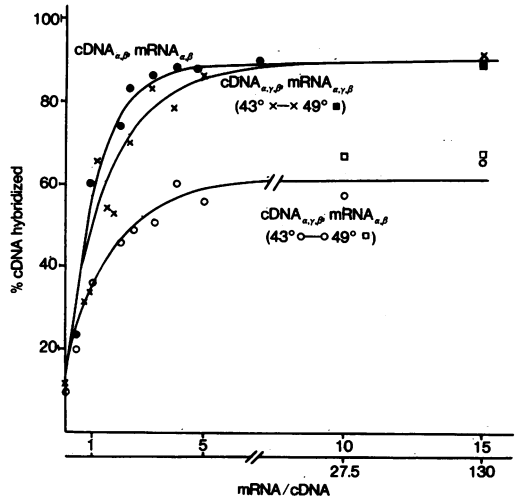


FIG. 2. Hybridization of human reticulocyte mRNA to human globin cDNA. One hundred twenty picograms (specific activity 7.5 × 10^6 cpm/μg of cDNA, calculated assuming dCTP to be 25% of the incorporated deoxynucleotides) of cDNA$_{\alpha,\beta}$ and cDNA$_{\alpha,\gamma,\beta}$ were hybridized to increasing amounts of human adult or fetal reticulocyte mRNA in 4 μl of hybridization buffer for 7 days at 43° or 49°. The RNA:cDNA ratios have been calculated from the amount of 9S RNA added, as calculated from polyacrylamide gel electrophoresis of poly(A)-containing RNA. ●, cDNA$_{\alpha,\beta}$ to mRNA$_{\alpha,\beta}$; ×, cDNA$_{\alpha,\gamma,\beta}$ to mRNA$_{\alpha,\gamma,\beta}$ at 43°; ■, the same at 49°; O, cDNA$_{\alpha,\gamma,\beta}$ to mRNA$_{\alpha,\beta}$ at 43°; □, the same at 49°.

total label incorporated by the crude cell-free system was recovered in the globin peaks, demonstrating the absence of significant amounts of other translatable mRNAs. Cytoplasmic mRNA from fetal liver was also tested in the cell-free system, and directed the synthesis of 52% α-globin, 8% β-globin, and 40% γ-globin.

Hybridization of cDNA to mRNA. Complementary DNAs prepared from reticulocyte mRNAs were hybridized to their own templates, and cDNA$_{\alpha,\gamma,\beta}$ (cDNA prepared from fetal reticulocyte mRNA$_{\alpha,\gamma,\beta}$, and containing these complementary sequences) was also hybridized to mRNA$_{\alpha,\beta}$ (Fig. 2). About 10% of the cDNA is S1-nuclease resistant in the absence of RNA, and a further 10–20% does not hybridize even at very high RNA:DNA ratios. These results are in agreement with

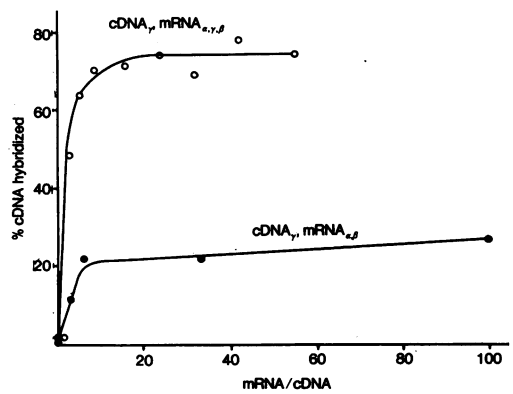


FIG. 3. Hybridization of γ-enriched cDNA to adult and fetal reticulocyte RNA. Forty picograms of γ-enriched cDNA were hybridized to adult reticulocyte (●) and fetal reticulocyte (O) mRNA for 21 days at 43°. Conditions were as in the legend of Fig. 2.

previously published data (9). The hybridization of cDNA$_{\alpha,\gamma,\beta}$ to mRNA$_{\alpha,\beta}$ proceeds to a plateau 65% of that for the homologous hybridization, indicating the absence of sequences in the mRNA complementary to certain sequences in the cDNA, presumably those for γ-globin mRNA. This is true both at high RNA:DNA ratio and at 49° as well as 43°.

The rate of reannealing of cDNA$_{\alpha,\beta}$ to mRNA$_{\alpha,\beta}$ at 1:1 ratios gave a $D_0t_{1/2}$ of 6 × 10^{-3}, corresponding to a complexity for the cDNA of 800 nucleotides by comparison with the figures determined for mouse globin mRNA (9, 10); for cDNA$_{\alpha,\gamma,\beta}$ the $D_0t_{1/2}$ is 7 × 10^{-3} and the complexity is 900 nucleotides. The reannealing rate was a unimodal sigmoid curve over 2 logarithm units, the hybridization going to 80–85% completion.

The cDNA from fetal reticulocyte mRNA (cDNA$_{\alpha,\gamma,\beta}$), which does not hybridize to mRNA$_{\alpha,\beta}$, is expected to be enriched in sequences homologous to mRNA$_\gamma$. This is confirmed by the fact that 75% hybridizes to fetal reticulocyte mRNA but only 20–25% to adult reticulocyte mRNA (Fig. 3). The amount of nonhybridizable material in the γ-enriched cDNA is higher than usual because of the purification, which also enriches for nonhybridizing sequences. The titration of this cDNA to adult reticulocyte mRNA varies with mRNA:cDNA ratio in the manner expected for a component annealing to an mRNA present at high concentration, and is almost certainly due to remaining contaminating cDNA sequences complementary to mRNA$_{\alpha,\beta}$.

Hybridization of cDNA to Nuclear RNA. HnRNA from bone marrow and fetal liver was isolated on sucrose gradients and then centrifuged on formamide–sucrose gradients at 20° (Fig. 4). The RNA sedimenting at greater than about 30 S was isolated.

cDNA$_{\alpha,\beta}$ and cDNA enriched for sequences complementary to γ-globin mRNA were hybridized to HnRNA fractions (>30 S) from marrow and fetal liver (Fig. 5).

Hybridization of cDNA$_{\alpha,\beta}$, cDNA$_{\alpha,\gamma,\beta}$, and cDNA$_\gamma$ (enriched for sequences complementary to γ-globin mRNA) to cytoplasmic bone marrow RNA and fetal liver RNA, fetal liver HnRNA (>30 S), and total fetal liver nuclear RNA were taken to completion at high RNA:cDNA ratios (Table 1).

DISCUSSION

In the wheat germ cell-free system, little if any γ-globin synthesis was detected in mRNA from adult reticulocytes, in agreement with studies *in vivo* demonstrating that γ-chain synthesis in adults represents at most 1% of the total globin synthesis (17). The ratio of γ-globin:β-globin synthesis with fetal mRNA was 2.5, within the expected range for cells from cord exchange transfusion blood. For reticulocyte mRNA, more than 80% of the total amino-acid incorporation was into globin. Cytoplasmic mRNA isolated from fetal liver directed the synthesis of γ-globin with little β-chain synthesis.

The cDNAs for both adult and fetal mRNAs are similar in size and hybridization behavior to those previously reported for mouse, rabbit, and duck globin mRNAs. Since the average sequence length of the cDNAs used is 300–350 nucleotides, the complexities obtained for cDNA$_{\alpha,\beta}$ (800 nucleotides) and cDNA$_{\alpha,\gamma,\beta}$ (900 nucleotides) demonstrate the absence of significant amounts of cDNAs derived from mRNAs other than those for globin, the major species present. The cDNA$_\gamma$ contains 70–75% sequence homologous to mRNA$_\gamma$, the contaminating sequences present being homologous to mRNA$_{\alpha,\beta}$.

TABLE 1. *Ratios of $mRNA_\gamma$, $mRNA_{\alpha,\beta}$, and $mRNA_{\alpha,\gamma}$ to total mRNA $\times 10^3$ for cytoplasmic and nuclear fractions from fetal liver and bone marrow*

	$mRNA_\gamma \times 10^3$/ total mRNA	$mRNA_{\alpha,\beta} \times 10^3$/ total mRNA	$mRNA_{\alpha,\gamma,\beta} \times 10^3$/ total mRNA	$mRNA_\gamma$/ total globin mRNA (%)
Cytoplasmic bone marrow mRNA	<0.09	1.6	Biphasic	<1.5–5
Cytoplasmic fetal liver mRNA	8.0	7.1	15.4	52
Bone marrow HnRNA	<0.008	0.08–0.16	—	<5
Fetal liver HnRNA	0.11	0.10	—	52
Total bone marrow nuclear RNA	<0.0033	0.33–0.5	—	<1

For each saturation curve of the type shown in Fig. 5, the ratio of RNA:cDNA (for cDNAs complementary to various globin mRNAs) that gave complete protection from S1 nuclease digestion was determined. From this value the proportion of each mRNA or HnRNA complementary to globin cDNAs was calculated. For bone marrow HnRNA it was not possible to obtain an RNA:cDNA ratio that would give an accurate value for the amount of $mRNA_\gamma$; the figure given ($<0.008 \times 10^{-3}$) is the maximum value obtained, with no correction for the contamination of $cDNA_\gamma$ with sequences homologous to $mRNA_\alpha$ or $mRNA_\beta$. For total bone marrow nuclear RNA the maximum RNA:cDNA ratio obtainable gave 50% of the total possible hybridization; for all other RNA samples, hybridization of the cDNA was essentially to completion. The cytoplasmic bone marrow mRNA was contaminated (as judged by electrophoretic gel analysis) with nucleic acids other than mRNA, and the absolute values for each RNA are approximately 4-fold lower than expected, but the ratio of $mRNA_\gamma$ to total globin mRNA is not affected.

The ratios of γ-globin mRNA to (α+β)-globin mRNAs of adult and fetal reticulocytes and bone marrow and fetal liver were determined by hybridization, and agreed with the ratios found by translation assay in the wheat germ cell-free system and the translation ratios found *in vivo* (24, 25). In the case of fetal liver cytoplasmic mRNA and HnRNA, there is equal hybridization to $cDNA_\gamma$ and to $cDNA_{\alpha,\beta}$ at low ratios, demonstrating that γ- and α-globin mRNA sequences are present in approximately equal amounts; the $cDNA_\beta$ component can be titrated only at higher RNA:cDNA ratios. There is twice as much hybridization to $cDNA_{\alpha,\gamma,\beta}$ as to $cDNA_\gamma$, as expected.

The equivalence of translation and mRNA ratios demonstrates that translational control is not a major control mechanism for globin synthesis in these cells, as cytoplasmic mRNA is not present other than in the proportions in which translation occurs *in vivo*.

Under our conditions of hybridization there was no evidence for cross-homology between β- and γ-mRNA sequences (26), as elevating the temperature of hybridization from 43° to 49° does not lower the proportion of double-stranded hybrid formed between $cDNA_{\alpha,\gamma,\beta}$ and $mRNA_{\alpha,\beta}$. The $cDNA_\gamma$, enriched for sequences complementary to γ-globin mRNA, was prepared from hybrid formed at the higher temperature.

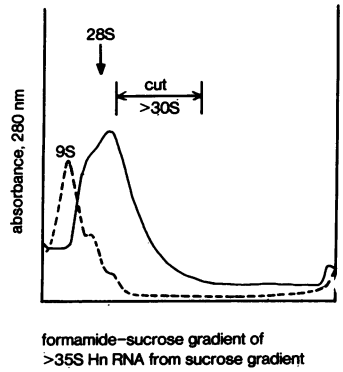


FIG. 4. Sedimentation profile of fetal liver HnRNA in formamide–sucrose gradient. The mouse globin mRNA sample used as a marker (dashed line) in a parallel tube was slightly contaminated with 18S and 28S ribosomal RNA. Sedimentation from left to right. The profile for bone marrow HnRNA was similar to that for fetal liver, with somewhat less material >35 S. Much of the absorbance is due to preribosomal RNA.

In experiments analyzing the level of mRNA sequences in nuclear RNA, one must always be mindful of the possibility of contamination by cytoplasmic or small nuclear mRNA sequences. This could arise either through fortuitous contamination due to gradient trailing or similar artifacts, or by noncovalent binding of mRNA molecules to long nuclear RNA sequences (12, 14, 27).

We believe that the possibility of fortuitous contamination can be eliminated by reconstruction experiments, in which mRNA of known size from one tissue (e.g., globin mRNA from reticulocytes) is mixed with nuclei from another, but similar, tissue that would not contain the mRNA, and the two extracted together (12, 14). This has been repeated as a control in these experiments, and less than 0.5% of the added globin mRNA was isolated with HeLa HnRNA from formamide–sucrose gradients (data not shown). This compared with 30% of nuclear globin mRNA found in HnRNA from bone marrow (Table 1). This 60-fold difference is far too great to be explained by gradient trailing or similar effects.

Noncovalent binding of small, presumably nuclear mRNA sequences to specific large nuclear RNA molecules is much harder to rule out, although reconstruction experiments demonstrate that such interactions do not occur with either

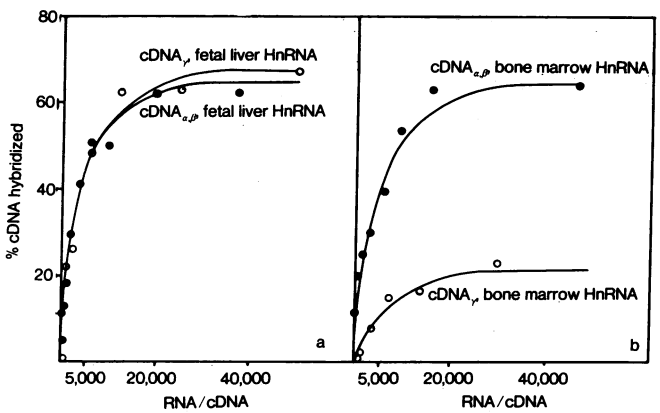


FIG. 5. Hybridization of $cDNA_{\alpha,\beta}$ and γ-enriched cDNA to fetal liver HnRNA and adult bone marrow HnRNA. (a) Forty picograms of $cDNA_{\alpha,\beta}$ (●) or γ-enriched cDNA (○) were hybridized to HnRNA >30 S, purified on a formamide gradient, 18 days, 43°. (b) As in a, but with adult bone marrow HnRNA.

cytoplasmic supernatants and heterologous nuclei, or purified mRNA and HnRNA (12, 14). The formamide gradient conditions used in our experiments were greater than 10° above the melting temperature of DNA of the same base composition. This makes it unlikely that globin mRNA could be associated with high molecular weight RNA by hydrogen bonding after formamide–sucrose gradient centrifugation, rather than being covalently linked. Similar conclusions were reached by Imaizumi *et al.* (11) and by MacNaughton *et al.* (28) for duck globin HnRNA after centrifugation on dimethylsulfoxide–sucrose or 85% formamide–sucrose gradients.

The high-molecular-weight RNA from fetal liver contains mRNA sequences for α-, β-, and γ-globins, while that from bone marrow contains the sequences for α- and β-globins and does not contain appreciable amounts of γ-sequences, as shown by the low hybridization values with $cDNA_\gamma$ even at high RNA:cDNA ratios.

The high-molecular-weight fraction (>30 S) of HnRNA from a formamide gradient sediments at about 35 S from the position of marker ribosomal RNAs, corresponding to an average molecular weight of 2×10^6, or 6000 nucleotides, 10 times the sequence length of globin mRNA (1, 2). The percentage of RNA sequences complementary to globin cDNA in marrow HnRNA is 0.01–0.015% and, thus, there is one globin sequence for α-chains and one for β-chains in about 50 HnRNA molecules >30 S on formamide gradients from adult bone marrow erythroid cells (10% of total normal marrow cells). This agrees with results published for duck globin HnRNA by Imaizumi *et al.* (11). It was not possible to obtain a high enough ratio of HnRNA:cDNA to titrate the γ-enriched cDNA with the small proportion (<1%) of γ-HnRNA present in adult erythroid tissue. However, by use of total nuclear RNA and cytoplasmic RNA it was shown that γ-globin mRNA sequences are present in both at the anticipated level of approximately 1% of $(\alpha+\beta)$-mRNA sequences (Table 1). This result confirms the absence of significant amounts of transcribed γ-globin mRNA sequences other than those processed and translated.

The data presented above demonstrate the absence of γ-globin mRNA sequences from HnRNA from adult bone marrow containing the expected amount of β-globin mRNA sequences. Thus, in a tissue in which approximately 2.5–5% of the total nonrepetitive sequences are transcribed (16, 29), genes that are extremely close in spatial terms are nonetheless not cotranscribed.

The simplest explanation of the ontogeny and genetics of the γ-, δ-, and β-globins is adjacent linkage on the chromosome, with no other coding genes interspersed. If this is the case, and making the further assumption that the mRNA is at the 3′-end of the HnRNA for this specific sequence as for HnRNA in general (15, 30), it can be stated that a denatured RNA molecule of sequence length $\geqslant 6000$ nucleotides containing $mRNA_\beta$ sequences does not contain $mRNA_\gamma$ sequences. This approach should permit a direct estimate of the intergene distance for closely linked genes such as the β-type globins.

We thank our colleagues, and in particular Drs. J. Paul, B. Young, and P. R. Harrison, for much useful advice. We also thank Prof. D. Weatherall, Dr. J. Clegg, and Dr. J. Pritchard of the Department of Haematology, University of Liverpool, for many helpful suggestions on human hematology. We are most grateful to the clinicians who cooperated with us in preserving suitable pathological material for molecular biological analysis. The Beatson Institute for Cancer Research is supported by the Medical Research Council and the Cancer Research Campaign. S. O. was on leave from the Institute of General Pathology, University of Milan, on a Royal Society Exchange Fellowship from the Accademia Nazionale dei Lincei.

1. Lingrel, J. B. (1972) in *Methods in Molecular Biology*, eds. Last, J. & Laskin, A. (Marcel Dekker, Inc., New York), Vol. 2, pp. 231–263.
2. Williamson, R., Morrison, M., Lanyon, G., Eason, R. & Paul, J. (1971) *Biochemistry* **10**, 3014–3021.
3. Verma, I. M., Temple, G. F., Fan, H. & Baltimore, D. (1972) *Nature* **235**, 163.
4. Ross, J., Aviv, H., Scolnick, E. & Leder, P. (1972) *Proc. Nat. Acad. Sci. USA*, **69**, 264–268.
5. Kacian, D. L., Spiegelman, S., Bank, A., Terada, M., Metafora, S., Dow, L. & Marks, P. A. (1972) *Nature* **235**, 167.
6. Harrison, P. R., Hell, A., Birnie, G. D. & Paul, J. (1972) *Nature* **239**, 219–221.
7. Bishop, J. O. & Rosbash, M. (1973). *Nature New Biol.* **241**, 204–207.
8. Packman, S., Aviv, H., Ross, J. & Leder, P. (1972). *Biochem. Biophys. Res. Commun.* **49**, 813–819.
9. Harrison, P. R., Birnie, G. D., Hell, A., Humphries, S., Young, B. D. & Paul, J. (1974) *J. Mol. Biol.* **84**, 539–554.
10. Young, B. D., Harrison, P. R., Gilmour, R. S., Birnie, G. D., Hell, A., Humphries, S. & Paul, J. (1974) *J. Mol. Biol.* **84**, 555–568.
11. Imaizumi, T., Diggelman, H. & Scherrer, K. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 1122–1126.
12. Williamson, R., Drewienkiewicz, C. E. & Paul, J. (1973) *Nature New Biol.* **241**, 66–68.
13. Stevens, R. H. & Williamson, A. R. (1972) *Nature* **239**, 143–146.
14. Ruiz-Carrillo, A., Beato, M., Schutz, G., Feigelson, P. & Allfrey, V. G. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3641–3645.
15. Darnell, J. E., Jelinek, W. R. & Molloy, G. R. (1973) *Science* **181**, 1215–1221.
16. Scherrer, K. & Marcand, L. (1968) *J. Cell. Physiol.* (Suppl. 1), **72**, 181–212.
17. Weatherall, D. J. & Clegg, J. B. (1972) *The Thalassaemia Syndromes* (Blackwell, London).
18. Huisman, T. H. J., Wrightstone, R. N., Wilson, J. B., Schroder, W. A. & Kendall, A. G. (1972) *Arch. Biochem. Biophys.* **153**, 850–853.
19. Aviv, H. & Leder, P. (1972) *Proc. Nat. Acad. Sci. USA*. **69**, 1408–1412.
20. Perry, R. P., LaTorre, J., Kelley, D. E. & Greenberg, J. R. (1972) *Biochim. Biophys. Acta* **262**, 220–226.
21. Roberts, B. E. & Paterson, B. M. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2330–2334.
22. Clegg, J. B., Naughton, M. A. & Weatherall, D. J. (1966) *J. Mol. Biol.* **19**, 91–108.
23. Busch, H. & Smetana, K. (1970) *The Nucleolus* (Academic Press, New York).
24. Wood, W. G. & Weatherall, D. J. (1973) *Nature* **244**, 162–165.
25. Nienhuis, A. W., Canfield, P. H. & Anderson, W. F. (1973) *J. Clin. Invest.* **52**, 1735–1745.
26. Forget, B. G., Benz, E. J., Jr., Skoultchi, A., Baglioni, C. & Housman, D. (1974) *Nature* **247**, 379–381.
27. Lane, C. D., Gregory, C. M., Iyazumi, T. & Scherrer, K. (1973) *Nature New Biol.* **243**, 78.
28. MacNaughton, M., Freeman, K. B. & Bishop, J. O. (1974) *Cell* **1**, 117–125.
29. Brown, I. R. & Church, R. B. (1972) *Develop. Biol.* **29**, 73–84.
30. Sheldon, R., Kates, J., Kelley, D. E. & Perry, R. (1972) *Biochemistry* **11**, 3829–3834.