Hemoglobin Synthesis in Somatic Cell Hybrids: Independent Segregation of the Human Alpha- and Beta-Globin Genes

Abstract. *Hybrid somatic cells containing a partial complement of human chromosomes were used to demonstrate that the human alpha- and beta-globin genes are located on different chromosomes. Two cell lines consisting of a cross of mouse with human fibroblasts contained the human alpha- but not the beta-globin gene, while a cross of human marrow cells with mouse erythroleukemia cells expressed the human beta- but not the alpha-globin gene.*

Generation of somatic cell hybrids of varying chromosomal composition has been useful for the analysis of both linkage relationships and the regulation of many structural genes (*1*). Hemoglobin contains polypeptides alpha and beta globin. We used two types of hybrid cells to obtain data indicating that the human globin genes are on separate chromosomes (asyntenic).

The first type, hybrid cell lines derived by fusion of human and mouse fibroblasts, contained most mouse and several human chromosomes. Hybridization of purified DNA from two cell lines to specific globin complementary DNA's (cDNA) served to demonstrate that the human α- but not the human β-globin gene was present in each. The second type, a hybrid cell line obtained by fusion of human marrow cells with mouse erythroleukemia (MEL) cells, contained only a few human chromosomes. The hybrid cells retained an important property of the MEL parent cells (*2*) because they could be induced to make hemoglobin by exposure to dimethylsulfoxide (Me_2SO). We have reported such a hybrid that contained human as well as mouse globin messenger RNA (mRNA) (*3*). Study of an additional hybrid line has allowed us to demonstrate independent or nonsimultaneous expression of the human α- and β-globin genes.

A human × mouse fibroblast hybrid (WAIIA) was obtained by fusion of the human fibroblast line, WI-38 (*4*), with the mouse line A-9, which is resistant to 6-thioguanine (*5*). Nine different human chromosomes were represented in these cells. The least frequent was present in 15 percent of the metaphase spreads, and the most frequent was present in 65 percent before and after expansion of the line for DNA extraction (*6*). The hybrid cells were grown in monolayer in F14 media supplemented with 10 percent fetal calf serum. Four weeks were required to expand the line to 5×10^9 cells.

DNA was extracted from the cells by a modification of the method of Marmur (*7*). Before hybridization the DNA was sonicated to an average length of 300 to 400 nucleotides. Human DNA was prepared from spleen and mouse DNA from embryos. Probes enriched in either human α- or β-globin cDNA sequences were prepared with messenger RNA extracted from human reticulocytes (*8*) as templates for a viral enzyme, RNA-directed DNA polymerase (*9*). Patients with homozygous β thalassemia have a deficiency of β-globin mRNA (*10*). Thus RNA prepared from their reticulocytes contains mainly α-globin mRNA and can be used to synthesize human α-globin enriched cDNA (*8*). Similarly, patients with Hb H disease have a deficiency of α-globin mRNA (*10*), permitting synthesis of β-globin enriched cDNA. Mouse globin mRNA was prepared from phenylhydrazine-induced reticulocytes (*8*). The conditions for synthesis of cDNA were as described (*8*), except that the concentration of deoxynucleotides was 50 μM, and a fraction of cDNA consisting of more than 300 base was recovered by centrifugation in an alkaline sucrose gradient. ^{32}P-Labeled deoxycytidine triphosphate (dCTP) was used to prepare a probe having a specific activity of 25,000 to 40,000 count/min per nanogram. To allow analysis of hybrid cell DNA for its human globin gene sequence content, the conditions of DNA · cDNA hybridization had to be sufficiently stringent to minimize reaction of the human probes with mouse globin gene sequences. Reactions performed in 50 percent formamide containing triple strength standard saline citrate (SSC) at 49°C provided satisfactory specificity (Fig. 1, A and B). Human DNA reacted equally with the α-globin enriched and β-globin enriched cDNA's.

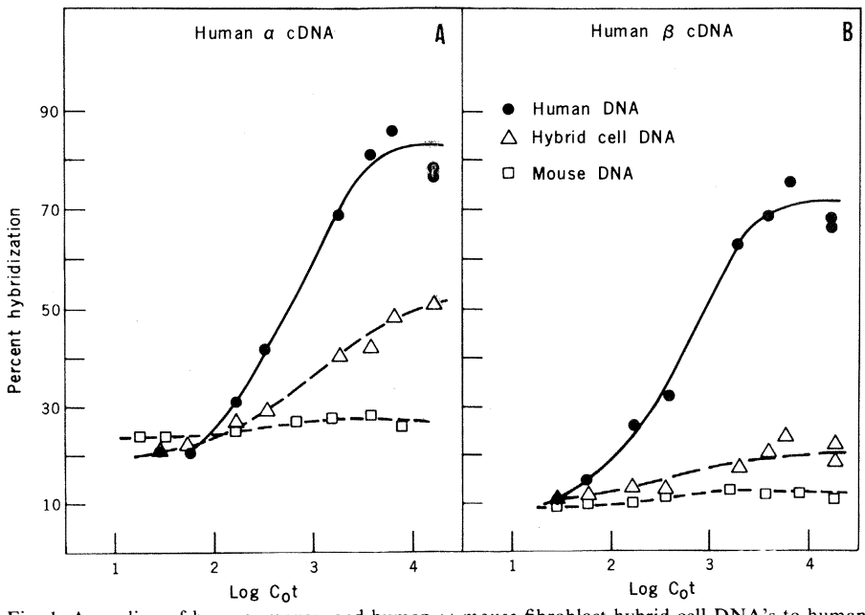

Fig. 1. Annealing of human, mouse, and human × mouse fibroblast hybrid cell DNA's to human α-globin enriched (A) and β-globin enriched (B) globin cDNA's. Analysis of each individual reaction was by batch hydroxylapatite chromatography (*19*). The concentration of DNA was 4.5×10^{-2} mole of nucleotide per liter. Each reaction contained 0.06 ng of cDNA; C_0t, moles of nucleotides per liter times the incubation time in seconds.

DNA from the hybrid cell line reacted significantly with human α-globin enriched cDNA but only minimally with the β-globin enriched cDNA (Fig. 1). The extent and apparent $C_0 t_{1/2}$ (concentration of nucleotides in moles per liter multiplied by the incubation time in seconds) of the reaction with the α-globin enriched cDNA may be interpreted to suggest that the concentration of the α-globin gene sequences in the hybrid DNA was approximately one-fourth that in normal human DNA (*11*). Assuming that each cell contains only one of each human chromosome, we can infer that a chromosome bearing the human α-globin gene was present in 50 percent of the hybrid cells, while that bearing the human β-globin gene was present in no more than 10 percent of the cells. The human globin gene sequence content of an additional hybrid cell line (J-10-H-12) (*6*) was determined by DNA-cDNA hybridization and similar results were obtained; α-globin sequences were present but β-globin sequences were not. Thus we have demonstrated independent segregation of the human α- and β-globin genes in two hybrid

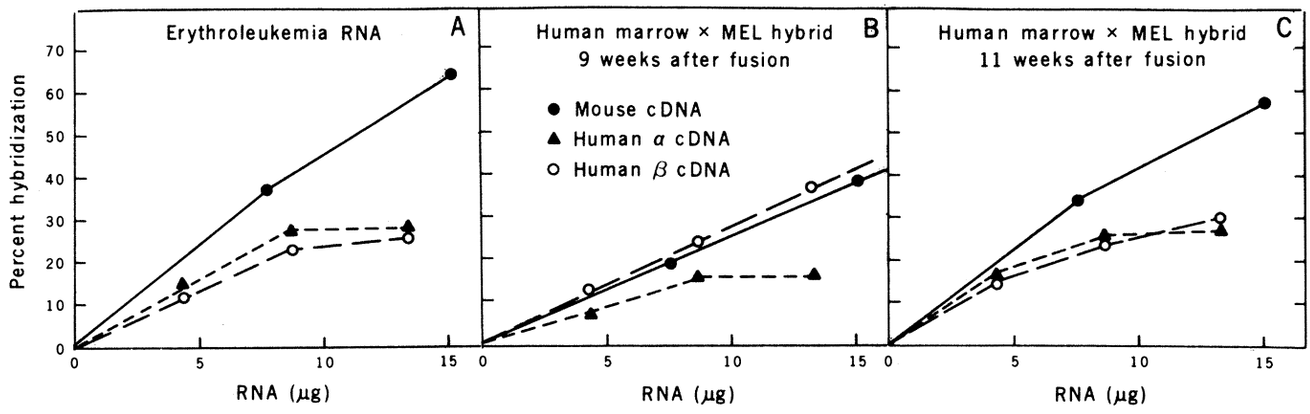

Fig. 2. Analysis of RNA extracted (A) from mouse erythroleukemia cells and (B and C) from a human marrow × MEL cell hybrid by annealing to globin cDNA.

cell lines, suggesting that these genes are on separate chromosomes.

To study the expression of the individual human globin genes in hybrid cells, we made fusions between human bone marrow and an MEL cell line resistant to 6-thioguanine (*3*). Fractionation of human bone marrow to obtain a cell population enriched in erythroid precursors, fusion of these cells to MEL cells, and growth of the resulting hybrid cells in selective media was as described (*3*). The hybrid nature of the cells was confirmed by demonstrating the heteropolymeric form (human-mouse) of glucose-6-phosphate dehydrogenase. Nine weeks after fusion sufficient hybrid cells (2×10^8) were available for RNA extraction. At this time, the cells contained on the average of three more biarmed chromosomes (five total) than did the MEL parent. After growth in 2 percent Me_2SO, 20 percent of the cells stained with benzidine (*12*) and thus contained hemoglobin. RNA was extracted from the intact cells as described (*8*). [^{3}H]dCTP was used to prepare cDNA's having a specific activity of 12,000 count/min per nanogram. The RNA · cDNA hybridizations were performed at 60°C in aqueous solution (*3*) and analyzed with micrococcal nuclease (*13*). Partial cross-hybridization of mouse mRNA to human cDNA occurring under these conditions (Fig. 2A) could not be prevented by performing the reactions in 50 percent formamide at 60°C (*7*).

The hybrid cells at 9 weeks after fusion contained human β-globin mRNA in addition to mouse globin mRNA (Fig. 2A). This conclusion is based on careful comparison between the reactions of hybrid cell RNA to the three probes with those of MEL cell RNA (Fig. 2, A and B). Mouse globin mRNA was present in lower concentrations in hybrid cell RNA as indicated by percent hybridization obtained at equivalent concentrations of RNA. Hybrid cell RNA reacted with the human α-globin probe only to the extent expected from cross-hybridization to mouse mRNA (compare Fig. 2, A and B). In contrast, hybrid cell RNA reacted with human β-globin cDNA to a slightly greater extent than with that of the mouse, indicating that human β-globin as well as mouse sequences were present. Two weeks later, after exposure to Me_2SO, only mouse globin mRNA sequences were found in hybrid cell RNA (Fig. 2C).

We interpret the lack of expression of the human α-globin gene at 9 weeks to be due to prior segregation of the chromosomes bearing that gene or genes. Subsequently, the chromosome bearing the β-globin gene was apparently lost since at 11 weeks the hybrid cells contained only one extra chromosome (compared to the MEL parent), and the β-globin gene was not expressed. We have now observed coexpression of mouse and either human or Chinese hamster globin genes in three separate hybrid cell populations [two previously reported (*3*)] obtained from a total of eight successful fusions, suggesting that coexpression is common in cells containing other than mouse chromosomes. Thus independent segregation of the human α- and β-globin genes seems the most likely explanation for their nonsimultaneous expression in the hybrid cells described in this report. Alternatively, both genes may have been present at 9 weeks, with only the β-globin gene being expressed. This hypothesis requires different mechanisms of regulation for the α- and β-globin genes. Study of additional hybrids will be necessary to adequately test this interesting possibility.

We used human × mouse somatic cell hybrids to obtain data that suggest that the human α- and β-globin genes are asyntenic. DNA from two separate hybrid lines was analyzed directly to determine which human globin gene or genes were present while the gene product, mRNA, was analyzed from another hybrid cell line. By linkage analyses the mouse α- and β-globin genes have been previously assigned to separate chromosomes (*14*). That the human α- and β-globin genes are not close together on the same chromosome was suggested by analyses of family pedigrees in which there were structural variants for both globins (*15*). The mutant proteins were found to segregate independently. Because frequently crossing-over occurs between homologous chromosomes during meiosis (*16*), this observation did not establish the asyntenic relationship of the genes. Price, Conover, and Hirschhorn (*17*) have reported that the human globin genes are located on chromosome 2 and a B-group chromosome. Because of technical problems, this result has remained controversial (*18*). Our results indicate that human α- and β-globin genes are asyntenic, and analysis of additional mouse × human cells hybrids may permit definitive assignment of the two human globin genes to particular chromosomes.

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Elimination of the Sarcoma Genome from Murine Sarcoma Virus Transformed Cat Cells

Abstract. *Cat cells transformed by Moloney murine sarcoma virus contain virus-specific sequences in their RNA and DNA. Cloned, spontaneous revertant cell lines derived from clones of these cells had no evidence of the sarcoma genome in the cell RNA or DNA as judged by RNA-complementary DNA or DNA-complementary DNA hybridizations. This is apparently the first report of loss of a transforming genome in a revertant cell line.*

Reversion of virus transformed cells is a rare event but has been observed in a number of systems including Rous sarcoma virus transformed hamster cells, polyoma and SV40 transformed hamster and mouse cells, and Moloney murine sarcoma virus (MSV) transformed mouse cells (*1*). Revertant cells can be obtained from every subclone of the sarcoma virus transformed mouse cells (*1*). This suggests that the capacity for reversion is inherent in all MSV transformed cells in culture. Clones of MSV transformed cat cells were obtained by the so-called "single-hit" infection of a cat cell line with the feline leukemia pseudotype of MSV [MSV(FeLV)] as described (*2*). The cloned 8C transformed cat cell line had a high rate of spontaneous reversion with loss of expression of murine p30 antigen and no rescuable sarcoma virus (*3*). This system was chosen because MSV contains murine leukemia virus (MuLV) sequences that are present in normal mouse cells, making revertant mouse cells more difficult to study (*4, 5*). We now report evidence for the loss of a transforming sarcoma genome in these revertant cells.

Because of the loss of the MuLV group-specific antigen of p30 in the revertants we began by examining the quantity of virus-specific RNA in these cells (*3*). With the use of an MSV and Moloney MuLV virus complex (*6*) (the ratio of biological activity of MSV to MuLV was 4 : 1) in an endogenous reverse transcriptase reaction with actinomycin D, a labeled DNA transcript complementary to $\geq$ 70 percent of the 65*S* viral RNA was made (Fig. 1a). This DNA was hybridized with cellular RNA, and the hybrids were analyzed by hydroxylapatite chromatography (*7*) by varying the cellular RNA and viral RNA concentrations in hybridizations of RNA and complementary DNA (cDNA). The 65*S* homologous viral RNA hybridized with 83 percent of the probe (cDNA) (Fig. 1a). The positive controls consisted of MSV transformed cat cell lines. Cell line P521 is a cloned MSV transformed cat cell line infected with and producing MSV and FeLV. The P521 cell RNA hybridized with 77 percent of the probe, with a half-saturation value (the RNA concentration of half-maximal hybridization with cDNA) of 10 μg of cell RNA per 0.1 ml of reaction mixture (Fig. 1a). Cell line 81 is a subclone of the 8C cat cell line transformed by MSV with rescuable MSV but no replicating ecotropic oncornavirus. Although its RNA also hybridized with 74 percent of the cDNA probe, higher concentrations of cell RNA were required (Fig. 1a). In the RNA-cDNA hybridization curves of Fig. 1a, the final degree of hybridization reflects the total genome content; the half-saturation value of RNA gives the copy number for the genome (Table 1). There were fewer copies of the viral genome in the cell line 81 RNA. The negative control was the normal cat strain CCC, the normal cat cell line from which all other lines were derived (*2*). Only 4 percent hybridized with the probe. The cat cell revertants 8CR4, 8CR7, and 8CR10 were derived from the

Table 1. Murine sarcoma–leukemia specific information in cat cell lines. The percentage of genome present as RNA is calculated as the maximal hybridization shown on the RNA-cDNA curve divided by the maximal hybridization of the probe with 65*S* homologous RNA (83 percent) minus the percentage of hybridization without RNA (4 percent) from each. The fraction of the cell RNA that is viral is the ratio of the half-saturation concentration of the viral RNA (0.03 μg/0.1 ml) to the half-saturation concentration of cell RNA. (The half-saturation viral RNA concentration was 0.03 μg/0.1 ml.) The percentage of genome present as DNA is the maximal hybridization on the DNA-cDNA curve divided by the maximal hybridization of probe with MSV-MuLV infected mouse cell DNA (80 percent). The number of viral copies per haploid genome is the $C_0t_{1/2}$ of the cell DNA reannealing divided by the $C_0t_{1/2}$ of the probe-cell hybridization (*20*). The percentage of genomes in the DNA and RNA are minimal values because the probe has murine leukemia–specific sequences not expected to hybridize with the DNA's and RNA's containing only MSV.

Cell line	Description	DNA genomes		RNA	
		In cell DNA (%)	Copies per haploid cell (No.)	In cell RNA (%)	Cell RNA that is viral (10^{-2} %)
P521	Cat cell line producing MSV(FeLV) and FeLV	72	16	92	30
81	MSV transformed cat cell line	38	3	89s	5
FeLV-81	FeLV infected 81 cell	64	8	85	6
CCC	Normal cat cell line	12	0	4	<.3
818	Revertant from 81	11	0	5	<.3
8CR7	Revertant from 8C	17	0	1	<.3
8CR4	Revertant from 8C	9	0	0	<.3
8CR10	Revertant from 8C	12	0	0	<.3
F49-1	Normal human amnion cell line	6	0		
Calf thymus	(Worthington)	2	0		
	No DNA	2	0		