serums are tested only with the proteins of modern creatures. By quantitatively comparing the specificities of these antiserums with those of antiserums to the purified proteins of living species, one can in theory determine the genealogical relations of extinct creatures to living ones. An attractive feature of the indirect method is that it focuses attention on those fossil molecules which have been least modified.

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Genes for Growth Hormone, Chorionic Somatomammotropin, and Growth Hormone–Like Gene on Chromosome 17 in Humans

Abstract. *The human genes for growth hormone* (GH), *chorionic somatomammotropin* (CSH), *and a third growth hormone–like gene* (GHL) *have been located on chromosome 17 in humans. DNA fragments of 2.6, 2.8, and 9.5 kilobase pairs containing* GH, CSH, *and* GHL, *respectively, were identified in human genomic DNA, and a 7.5-kilobase DNA fragment related to growth hormone DNA sequences was found in mouse cells. In somatic hybrids of human and mouse cells containing reduced numbers of human chromosomes, but a normal complement of mouse chromosomes, the mouse 7.5-kilobase DNA fragment was always present, whereas the 2.6-, 2.8-, and 9.5-kilobase human fragments were present only when human chromosome 17 was also present.*

The human polypeptide hormones growth hormone and chorionic somatomammotropin (also called placental lactogen) (*1*) are produced in the pituitary and placenta, respectively, yet they are closely related. They are composed of 191 amino acid residues and show about 85 percent amino acid-sequence homology (*2*). Their messenger RNA's (mRNA's) have more than 90 percent homology (*3, 4*). The human genes coding for growth hormone and chorionic somatomammotropin have been cloned, and DNA sequence analysis indicates that the genes have similar intervening sequences (*5*).

A second pituitary hormone, prolactin, shares to a lesser degree a structural homology with growth hormone and chorionic somatomammotropin (*6*). All three polypeptide hormones are believed to originate from a common ancestral gene by gene duplication (*4, 7, 8*) and thus may be closely linked on the chromosome.

The growth hormone gene (*GH*), the chorionic somatomammotropin gene (*CSH*), and a third growth hormone–like gene (*GHL*) are located on human chromosome 17. We used somatic cell hybrids of human and mouse cells to determine these locations. Cell hybrids were constructed and maintained by methods previously described (*9–11*); they contain a normal complement of mouse chromosomes, but the human complement contains reduced numbers and different combinations of human chromosomes. Cell hybrids containing 3 to 22 different human chromosomes were examined (Table 1).

Large-molecular-weight DNA was isolated from human T cell lymphoblasts, from the RAG mouse cell line, and from human-mouse cell hybrids by the method previously described (*11*). DNA (10 to 20 μg) was then digested to completion by the restriction endonuclease Eco RI. Digested fragments were separated by electrophoresis through a 1 percent agarose gel and transferred to nitrocellulose filters by the method of Southern (*12*).

The construction of the recombinant plasmid chGH800/pBR322, which contains DNA complementary to nearly full-length human growth hormone mRNA, has been previously discussed (*4*). The 800-nucleotide growth hormone complementary DNA (cDNA) insert was isolated from pBR322 plasmid DNA by Hind III digestion and preparative gel electrophoresis (*4*). A ^{32}P-labeled probe was made by labeling the insert by the method of random priming with calf thymus DNA primers (*13*). Specific activities greater than 10^8 cpm/μg were

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Table 1. Human chromosome distribution in human-mouse cell hybrids segregating *GH*, *CSH*, *GHL*, and Galk. Human-mouse cell hybrids were isolated (*9*) by use of chromosomally and enzymatically characterized parental cell lines. Cell hybrids derived from chromosomally normal human parental cell lines were WIL hybrids (WI-38 × mouse LM/TK$^-$), RAS (mouse RAG × human SH 421), and SIR hybrids (human GM 469 × mouse RAG) (*9, 27, 28*). The human cell lines preceded by GM were obtained from the Human Genetic Mutant Cell respository, Camden, N.J. The other hybrids were isolated from human cell lines with chromosome translocations. Some TSL (GM 2808 × LM/TK$^-$) hybrids segre-

Hybrid*	*GH** (2.6)	*CSH*† (2.8)	*GHL*‡ (9.5)	Galk§	Chromosome¶									
					1	2	3	4	5	6	7	8	9	10
WIL-2	+	+	+	+	−	−	−	−	−	−	−	+	−	+
WIL-8Y	+	+	+	+	−	−	−	−	−	+	+	−	−	+
TSL-2	+	+	+	+	−	+	−	−	+	+	−	−	−	+
TSL-5	+	+	+	+	−	−	−	+	+	−	−	−	−	+
TSL-6	+	+	+	+	−	−	−	−	+	−	−	−	−	+
TSL-8	+	+	+	+	−	−	−	−	−	−	−	−	−	−
RAS-8	+	+	+	+	+	+	+	+	+	+	+	+	−	+
RAS-9DT	+	+	+	+	+	+	+	+	−	−	+	+	+	+
SIR-4	−	−	−	−	−	−	−	−	+	+	+	+	−	+
SIR-8	+	+	+	+	+	+	+	+	+	−	+	+	+	+
XTR-3BSAgA	+	+	+	+	−	−	−	+	+	+	+	+	+	+
DUA-5	+	+	+	+	−	−	+	−	+	−	−	−	−	−
DUM-13	+	+	+	+	+	+	+	+	−	+	+	+	−	+
ALR-2	+	+	+	+	+	−	+	+	+	+	+	−	+	+
JSR-6C	−	−	−	−	−	+	+	−	+	−	−	−	−	+
JSR-17G	+	+	+	+	+	−	+	−	+	−	−	−	−	+
JSR-24D	+	+	+	+	+	−	+	−	+	+	−	+	−	+

*The growth hormone gene (*GH*) was measured as the 2.6-kb fragment (*5*). †The chorionic somatomammotropin gene (*CSH*) was identified as the 2.8-kb fragment (*5*). ‡The growth hormone-like gene (*GHL*) is detected in the 9.5-kb fragment. §Galk, a chromosome 17 marker, was analyzed by the method previously described (*11*). ¶In each hybrid, chromosomes have been determined by Giemsa-trypsin staining (*32*) and the Paris system of human nomenclature (*34*) is used. In

routinely obtained. The hybridization with ^{32}P-labeled probe of filters containing human, mouse, and human-mouse cell hybrid DNA fragments and washing of the filters to remove nonspecifically bound probe are described in Fig. 1.

In the control mouse line, the human growth hormone probe hybridized with a single DNA fragment of 7.5 kb (Fig. 1, channel A). Since the human growth hormone and chorionic somatomammotropin mRNA's have greater than 90 percent homology, either the mouse *GH* and *CSH* genes are contained within a common Eco RI fragment of 7.5 kb or fragments of similar size, or the growth hormone probe detects only one of the two mouse genes. In the control human cell line, the ^{32}P-labeled human growth hormone probe hybridized with three DNA fragments of 9.5, 2.8, and 2.6 kb (Fig. 1, channel B). In experiments with *GH* and *CSH* genes cloned from human DNA, it has been demonstrated that the 2.6-kb fragment contains the *GH* gene, and the 2.8-kb fragment contains the *CSH* gene (*5*) (see also Fig. 1, channel B). Recently, Goodman *et al.* (*14*) described two types of *GH* gene sequences, each contained within Eco RI restriction fragments of 2.6 kb, and three types of *CSH* gene sequences, all contained within 2.8-kb fragments. The variant *GH* and *CSH*

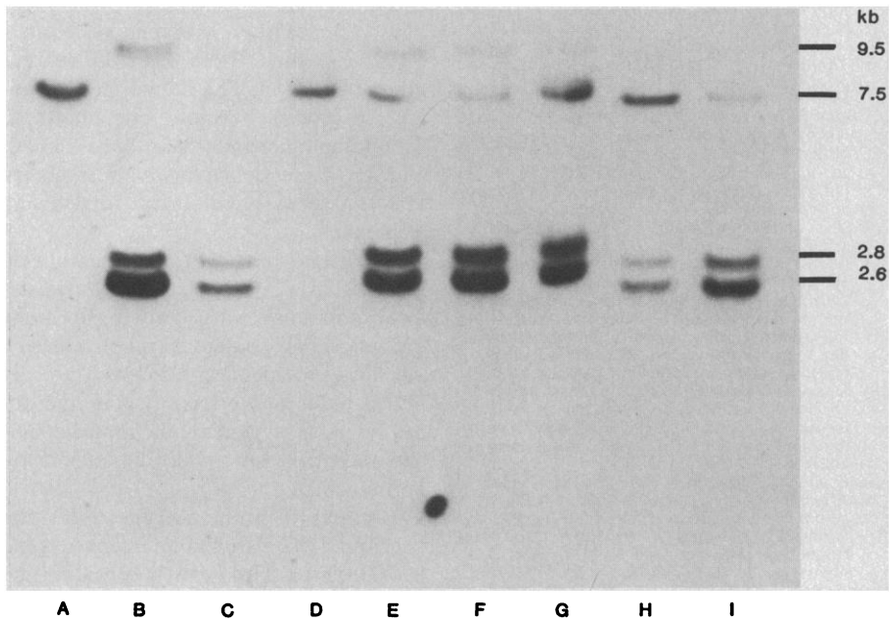

Fig. 1. Analysis of human and mouse growth hormone-related DNA fragments present in Eco RI restriction endonuclease digests of mouse, human, and human-mouse cell hybrid DNA. DNA isolation, digestion, and transfer to filters are described in the text. Before hybridization, nitrocellulose filters were treated for 2 days at 42°C in a 10-ml mixture of 50 percent deionized formamide, fivefold-strength SSC (0.75*M* NaCl, 75 m*M* trisodium citrate), fivefold-strength Denhardt's reagent (*25*), 50 m*M* sodium phosphate buffer (*p*H 6.5), and sonicated denatured salmon sperm DNA (Sigma) (500 μg/ml). Filters were then hybridized overnight at 42°C in a 10-ml solution consisting of 50 percent deionized formamide, fivefold-strength SSC, regular-strength Denhardt's reagent, 20 m*M* sodium phosphate buffer (*p*H 6.5), sonicated denatured salmon sperm DNA (200 μg/ml), 10 percent sodium dextran sulfate 500 (Pharmacia) (*26*), and ^{32}P-labeled growth hormone probe (5×10^6 count/min). Filters were washed in one-tenth strength SSC and 0.1 percent sodium dodecyl sulfate at 50°C. Labeled DNA bands were detected by exposing the filters to x-ray film for 1 to 5 days in the presence of an intensifying screen (DuPont). The growth hormone-related DNA patterns are shown for (A) mouse RAG cells, (B) human T cell lymphoblasts, (C) hybrid DUA-5, (D) hybrid JSR-6C, (E) hybrid TSL-8, (F) hybrid TSL-5, (G) hybrid XTR-3BSAgA, (H) hybrid JSR-17G, and (I) hybrid TSL-2. Fragments of Hind III-digested lambda DNA (New England BioLabs) were used as molecular weight markers. The 9.5-kb fragment is clearly present in all cell hybrids except JSR-6C (D) and SIR-4 (not shown), when scored from the original autoradiogram.

gate the 17qter→17p13::p21→3pter translocation (*29*). The XTR hybrid (GM 2899 × RAG) has not retained its [46,X,t(X;3) (q26;q13)] translocation (*30*) after counterselection on 8-azaguanine-supplemented medium. DUA (DUV × A9) and DUM (DUV × RAG) hybrids involve the [46,X,t(X;15) (p11;q11)] rearrangement, which segregates the Xqter→Xp11::15q11→15qter and 15pter→15q11::Xp11→Xpter translocations (*31*). ALR hybrids (AnLy × RAG) segregate the Xqter→Xq12::9p24→9qter rearrangement (*32*). The JSR hybrids (JoSt × RAG) were obtained from human cells with a [46,XX,t(7:9) (q22;p24)] rearrangement and segregates the 7pter→7q22::9p24→9pter translocation (*33*).

Chromosome‖																		
11	12	13	14	15	16	17	18	19	20	21	22	X	Y	17/3	9/X	7/9	X/15	15/X
−	+	−	−	+	−	+	−	−	−	+	−	+	−					
+	−	−	+	−	−	+	+	−	+	+	−	+	−					
−	+	−	−	−	−	−¶	+	−	+	+	−	+	−	+¶				
+	+	−	−	−	−	+	+	−	−	+	−	−	−					
−	−	−	−	−	−	−¶	−	+	−	−	−	−	−	+¶				
−	−	−	−	−	−	+	+	−	+	−	−	−	−					
+	+	+	+	+	+	+	+	+	+	+	+	+	−					
+	+	−	+	+	+	+	+	+	+	+	+	+	−					
+	−	+	+	+	+	−	−	+	+	+	−	+	−					
+	+	+	+	+	+	+	+	+	−	+	+	+	+					
−	−	−	+	−	−	+	+	+	−	+	−	−	−					
+	−	−	+	+	−	+	+	−	−	+	−	−	−				+	+
+	−	+	−	+	+	+	+	+	+	+	+	+	−				+	
+	+	+	+	+	+	+	−	+	+	+	−	−	−		+			
−	−	−	+	+	+	−	+	−	−	−	−	+	−			+		
−	+	−	+	+	−	+	+	−	+	+	−	−	−			+		
+	−	+	+	+	+	+	+	+	+	+	−	+	−			+		

addition enzyme markers whose genes have been assigned to each human chromosome, except the Y, have been tested on each hybrid and confirm the chromosome analysis (data not shown) (*10, 27, 35*). ¶In TSL hybrids not retaining a normal chromosome 17, the 17/3 translocation was present instead. Virtually all of chromosome 17 is involved in this rearrangement.

genes differ by only a few nucleotides each (*14*) and would not be resolved in our experiments (Fig. 1, channel B). The 9.5-kb fragment, which also hybridized with the ^{32}P-labeled human growth hormone probe (*5*; Fig. 1, channel B), has not been further characterized.

Since there is only 16 percent homology between human growth hormone and prolactin amino acid sequences (*6*), it is unlikely that prolactin sequences would be detected by the growth hormone probe under the conditions used for hybridization in these experiments (Fig. 1). The mouse 7.5-kb fragment hybridized to a greater degree with the human probe than the human 9.5-kb fragment did (Fig. 1). Since rat and human growth hormone mRNA have 76 percent homology (*8*), the growth hormone DNA sequences contained within the 9.5- and 2.6-kb fragments probably differ by more than 25 percent. Thus the 9.5-kb gene is not an allele of the 2.6-kb *GH* gene; it is more likely that it contains another gene related to human *GH*. We have designated this gene the growth hormone-like (*GHL*) gene. Similar phenomena have been described in other gene systems. For example, genes related to the ovalbumin gene have been characterized in the chicken (*15*).

The mouse 7.5-kb DNA fragment was present in all cell hybrids, and the 2.6-, 2.8-, and 9.5-kb human fragments were present in all hybrids except in JSR-6C and SIR-4, in which all three were absent (see Fig. 1 for representative data). Since the mouse sequences served as an internal hybridization control, the absence of the human DNA fragments was not due to factors such as poor DNA transfer from the agarose gel, loss of DNA from the nitrocellulose filter, or poor hybridization. Therefore the absence must result from a loss, in these hybrids, of the human chromosomes containing the hybridizing sequences.

The cell hybrids were analyzed for their human chromosome complement by analysis of enzyme markers for each of the 22 different human autosomes and the X chromosome and also by direct analysis of the chromosomes (see Table 1). The 9.5-, 2.8-, and 2.6-kb fragments segregated concordantly with chromosome 17 and the chromosome 17 enzyme marker galactokinase (Galk) (Table 1). Fifteen of the 17 hybrids retained chromosome 17 and Galk. The high retention of this chromosome was in part due to the selection for human chromosome 17 in six of the hybrids whose mouse parental genome was derived from the thymidine kinase-deficient LM/TK$^-$ line; the hybrids were WIL-2, WIL-8Y, and TSL-2, TSL-5, TSL-6, and TSL-8 (Table 1). Since these hybrids were maintained on the HAT (hypoxanthine, aminopterin, thymidine) selection media, human chromosome 17, which contains the thymidine kinase gene, must be retained for cell growth (*16*). No human chromosome or enzyme marker was retained in all six cell hybrids except chromosome 17, Galk, and the 9.5-, 2.8-, and 2.6-kb DNA fragments. All other chromosomes segregated independently, an indication that the sequences hybridizing with the growth hormone probe are located on chromosome 17. Hybrids TSL-2 and TSL-6 did not have a normal chromosome 17, but they did retain a 17/3 translocation(17qter → 17p13::3p21 → 3pter) that contains an almost intact chromosome 17; these hybrids also retain the 9.5-, 2.8-, and 2.6-kb fragments (Table 1). Cosegregation of the three fragments with this chromosome 17 translocation provides further support for the localization on chromosome 17. Hybrids TSL-6 and TSL-8 contained only a few human chromosomes, yet they also retained the 9.5-, 2.8-, and 2.6-kb fragments. The only human chromosome in common was chromosome 17.

The 11 remaining hybrids were obtained without selection for chromosome 17. Hybrids JSR-6C and SIR-4 lost human chromosome 17 and concurrently lost Galk and the 9.5-, 2.8-, and 2.6-kb fragments (Table 1; Fig. 1). All other hybrids retained chromosome 17, Galk, and the 9.5-, 2.8-, and 2.6-kb fragments (Table 1). The presence of the 9.5-, 2.8-, and 2.6-kb fragments (Table 1) is not correlated with the presence of any other human chromosome in these 11 hybrids.

In all cell hybrids studied, the correlation of the 9.5-, 2.8-, and 2.6-kb fragments with human chromosome 17 and Galk, and the absence of correlation with any other human chromosome, demonstrates that the *GH*, *CSH*, and *GHL* genes are located on human chromosome 17. By determining the chromosome locations for closely related genes,

one can obtain insight into chromosome evolution. It is known that closely related genes, derived from a common ancestral gene, do not have to be located on the same chromosome. For example, the α- and β-globin genes are located on chromosomes 16 and 11, respectively (*17, 18*). However, it is estimated that α- and β-globin genes have diverged from this common ancestral gene approximately 500 million years ago (*19*). The γ-, δ-, and β-globin genes are closely linked on the short arm of human chromosome 11 (*20–22*). These genes have diverged much more recently (*19*). Other related genes, such as salivary and pancreatic amylase, are also closely linked on chromosome 1 in humans (*23, 24*). It is estimated that the *GH* and *CSH* genes diverged approximately 50 million to 60 million years ago (*8*). As shown here, they have retained chromosomal linkage. The prolactin and growth hormone genes diverged approximately 400 million years ago (*8*). With a probe for the human prolactin gene it would be possible to determine whether this gene is linked to *GH*, *CSH*, and *GHL*. Whether the genes are contiguous on the DNA of chromosome 17 is an issue that must be resolved by other methods, for example, by cloning of large DNA fragments.

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Strain Dependence of the Antiproliferative Action of Interferon on Murine Erythroid Precursors

Abstract. *Electrophoretically pure mouse interferon inhibits erythropoietin-dependent proliferation of committed erythroid precursors (CFU-E) obtained either from adult mouse bone marrow or from 14-day fetal mouse livers. The degree of inhibition is significantly influenced by the genotype of the cell donor; about ten times as much interferon is required to inhibit proliferation of CFU-E from C57BL/6 than is needed for comparable inhibition of CFU-E from BALB/c or Swiss mice. These strain-dependent results point to the existence of genes that influence the degree of the inhibitory effect of interferon on cell multiplication.*

The committed erythroid precursors (CFU-E) of mammalian erythropoietic tissues are the target cells for erythropoietin [erythropoiesis-stimulating factor (ESF)] and depend strictly on the hormone for their proliferation and differentiation. When CFU-E are cloned in vitro in the presence of ESF (*1*), they give rise in 48 hours to erythroblastic colonies of 8 to 40 elements. This hormone-dependent system is sensitive to the antiproliferative effect of interferon preparation, as has bee observed in cultures of murine adult bone marrow (*2*) and fetal liver (*3*). However, because interferon represented at best only 0.1 percent of the protein in the preparations used for these studies, it was important to reexamine the effect of CFU-E of using electrophoretically pure mouse interferon, which has recently become available (*4*).

It was our aim to determine the dose-response curve relating concentration of electrophoretically pure interferon and the degree of inhibition of the growth of normal mouse femoral CFU-E. Our findings confirm the specific antiproliferative action of pure interferon on mouse CFU-E and support the new concept of a strain-to-strain variation in the sensitivity of mouse CFU-E to interferon. By use of a slight modification of the techniques of McLeod *et al.* (*5*), femoral bone marrow from male 8- to 10-week-old C57l-6, B10D2, Swiss, or BALB/c mice was cultured for the growth of erythropoietic colonies in plasma clots. Briefly, in any 1 ml of final mixture, 1×10^5 to 1.5×10^5 nucleated cells were suspended in supplemented Dulbecco's modified Eagle medium (DMEM) enriched with fetal calf serum, bovine serum albumin, antibiotics, and bovine citrated plasma. For each experiment, three or four culture samples containing a standard dose of 0.3 IRP unit of ESF were supplemented with a dose of pure interferon, graded from 5 to 500 antiviral units, delivered in 250-μl portions after dilution in DMEM. Controls were one sample without ESF or interferon and one sample with ESF only, both supplemented with 250 μl of diluted interferon buffer. Just after addi-

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