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The Prolactin Gene Is Located on Chromosome 6 in Humans

Abstract. *The gene for prolactin has been located on chromosome 6 in humans. DNA fragments of 4.8 and 4.0 kilobases containing prolactin gene sequences were identified in human genomic DNA, whereas DNA fragments of 7.4, 3.6, and 3.3 kilobases containing prolactin gene sequences were found in mouse cells. In somatic cell hybrids of human and mouse cells the 7.4-, 3.6-, and 3.3-kilobase mouse fragments were always present, whereas the 4.8- and 4.0-kilobase human fragments were only present when human chromosome 6 was also present. We conclude that the prolactin gene resides on chromosome 6, a different location from those of the genes for the related hormones chorionic somatomammotropin and growth hormone.*

The polypeptide hormones growth hormone, chorionic somatomammotropin (placental lactogen), and prolactin belong to a common family (*1, 2*). Human growth hormone and chorionic somatomammotropin are composed of 191 amino acid residues and are closely related, with about 85 percent homology in their amino acid sequences (*3, 4*). Human prolactin is composed of 199 amino acid residues and is more distantly related, having only 26 percent homology with human growth hormone and 27 percent homology with chorionic somatomammotropin (*4*). The human growth hormone and human chorionic somatomammotropin messenger RNA's (mRNA's) have 92 percent homology, whereas the human prolactin mRNA has 42 percent homology with human growth hormone and 41 percent homology with chorionic somatomammotropin sequences (*4–6*). The genes coding for these three polypeptide hormones are believed to have originated from a common ancestral gene by duplication. Prolactin is thought to be the oldest member of this group (*4, 7, 8*). While the human growth hormone, chorionic somatomammotropin, and a third growth hormone–like gene have been located on chromosome 17 (*9*), the chromosome localization of the human prolactin gene (*prl*) was not known.

We report here that the prolactin gene is not on the same chromosome as the growth hormone gene, the chorionic somatomammotropin gene, and the growth hormone–like gene; rather it is located on chromosome 6 in humans. Somatic cell hybrids of human and mouse cells containing all the mouse chromosomes but lacking certain human chromosomes have been used to determine this chromosome localization. These cell hybrids were constructed, maintained, and analyzed as previously described (*10*).

Large-molecular-weight DNA was isolated from cultured mouse cells, human cells, and human-mouse cell hybrids and was digested with the restriction endonuclease Eco RI. The digested fragments were then separated by electrophoresis on agarose gels and transferred to nitrocellulose filters (*9, 11*). Restriction fragments containing the prolactin genes were analyzed by hybridizing the filters with labeled prolactin complementary DNA (cDNA) probes. The cloning of DNA complementary to both rat and human prolactin mRNA has been reported (*4, 12*). ^{32}P-Labeled prolactin probes were prepared by nick translation or by random priming with calf thymus DNA primers (*9, 11*). Specific activities greater than 10^8 cpm/μg were routinely obtained. The hybridization of these probes with filters containing human, mouse, or human-mouse cell hybrid Eco RI restriction fragments and the washing of the filters to remove nonspecifically bound probe were performed as described (*9, 11*).

In the control mouse line, the rat prolactin probe hybridized with three fragments of 7.4, 3.6, and 3.3 kb (Fig. 1, channel i). The rat prolactin probe did not cross-hybridize with human DNA under these hybridization and washing conditions (Fig. 1, channel j). Similarly, the human prolactin probe did not cross-hybridize with mouse DNA sequences (Fig. 1, channel a). In the control human cell line and in all the human cell lines used in this investigation, the human prolactin probe hybridized with two fragments of 4.8 and 4.0 kb (Fig. 1, channel b).

The mouse 7.4-, 3.6-, and 3.3-kb DNA fragments were present in all cell hybrids, while the human 4.8- and 4.0-kb

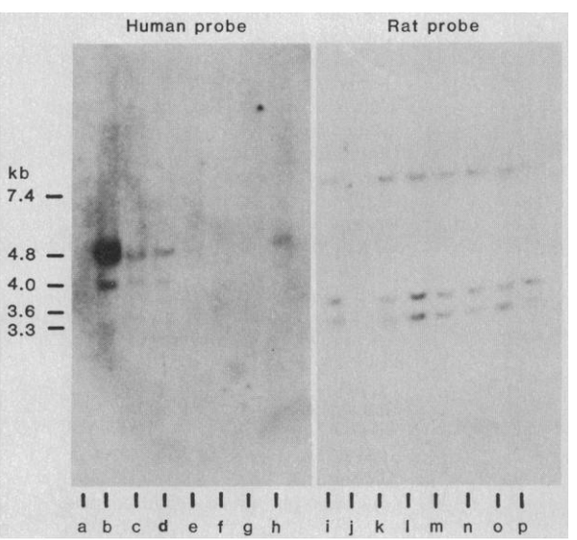

Fig. 1. Analysis of human and mouse prolactin DNA sequences present in Eco RI restriction digests of mouse, human, and human-mouse cell hybrid DNA. Isolation, digestion, and transfer of DNA to filters and hybridization were as described (*1, 11*). Labeled DNA bands were detected by exposing the filters to x-ray film for 2 to 10 days in the presence of an intensifying screen (Dupont Lightning Plus). Channels a through h were hybridized with human prolactin probe, while channels i through p were hybridized with rat probe. Prolactin hybridization patterns are shown for (channels a and i) mouse RAG cells; (channels b and j) human T cell lymphoblasts; (channels c and k) hybrid ALR-2; (channels d and l) hybrid TSL-2; (channels e and m) hybrid TSL-5; (channels f and n) hybrid TSL-6; (channels g and o) hybrid WIL-3; and (channels h and p) hybrid WIL-6. Fragments of Hind III–digested lambda phage DNA (New England Biolabs) were used as markers for molecular size. Hybrids ALR-2, TSL-2, and WIL-6 contained the human prolactin DNA sequences.

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DNA fragments were present in only 6 of 16 cell hybrids examined (Fig. 1).

The cell hybrids were analyzed for their human chromosome complement by analysis for enzyme markers specific for each of the 22 different human autosomes and the X chromosome (see Table 1). The 4.8- and 4.0-kb DNA fragments that hybridized to human prolactin cDNA segregated concordantly with malic enzyme 1 and superoxide dismutase 2, the chromosome 6 enzyme markers (Table 1). No other enzyme markers could be correlated with the presence of the 4.8- and 4.0-kb human prolactin DNA fragments (Table 1).

Eight cell hybrids were analyzed by Giemsa-trypsin staining of their chromosomes (*13*). These hybrids were WIL-2, WIL-8X, WIL-10S, TSL-2, TSL-5, TSL-6, ALR-2, and DUA-5 (see legend to Table 1). Of these eight hybrids, TSL-2 and ALR-2 retained chromosome 6 and the DNA fragments hybridizing to human prolactin cDNA, while the other six hybrids did not retain either human chromosome 6 or the human prolactin DNA sequences. The presence of the 4.8- and 4.0-kb human prolactin DNA fragments always correlated with the presence of human chromosome 6 and the human chromosome 6 enzyme markers. The presence of all other human chromosomes could not be correlated with the presence of the 4.8- and 4.0-kb human prolactin DNA fragments. This demonstrates that the prolactin gene is located on chromosome 6 in humans.

The prolactin gene is thus located on a different chromosome from that of the related growth hormone and chorionic somatomammotropin genes, which are both located on human chromosome 17 (*9*). A similar situation has occurred within the globin gene family. The different α-globin genes are located on human chromosome 16 while the different β-globin genes are located on human chromosome 11 (*14, 15*). We have recently found that the genes coding for leukocyte and fibroblast interferon are both located on chromosome 9 in humans (*16*).

Table 1. Segregation of the prolactin gene (*prl*) with enzyme markers in human-mouse cell hybrids. Cell hybrids were derived from WIL (WI-38 × mouse LM/TK$^-$), TSL (GM2808 × LM/TK$^-$), DUA (DUV × mouse A9), ALR (AnLy × mouse RAG), JSR (JOST × RAG), MAR (GM654 × RAG), and REW (WI-38 × RAG) (*10, 13, 17*). Human chromosomes retained were determined with the use of enzyme markers (*18, 19*). Chromosome localizations of genes coding for the enzyme markers have been documented (*19*). The concordant segregation column tabulates the number of hybrid cell lines that retained a specific human chromosome (as determined by enzyme markers whose genes have been assigned to each human chromosome) and also the human prolactin hybridizing sequences, or lost both the chromosome and human prolactin sequences. The discordant segregation column shows the number of hybrid cell lines which retained either the human chromosome or human prolactin sequences but not both. Human prolactin hybridizing sequences could be detected when approximately 15 percent of a hybrid cell line contained human chromosome 6. Since some enzyme markers could be detected when only 5 to 10 percent of a hybrid line contained the corresponding human chromosome, those hybrids with faintly observable enzyme marker activity were excluded from the concordant-discordant tabulations. This and the lack of enough cell hybrid homogenate for testing all enzyme markers accounts for instances where less than 16 hybrids were tabulated. The scoring of *prl*, enzyme markers, and chromosomes were determined on the same cell passage.

Chromosome	Enzyme marker	Prolactin	
		Concordant	Discordant
1	Adenylate kinase 2/peptidase C	10	5
2	Isocitrate dehydrogenase 1/malate dehydrogenase 1	9	7
3	Aminoacylase 1	9	7
4	Peptidase S	7	8
5	Hexosaminidase B	10	6
6	Malic enzyme 1/superoxide dismutase 2	16	0
7	β-Glucuronidase	6	7
8	Glutathione reductase	9	6
9	Adenylate kinase 1	9	6
10	Glutamate oxaloacetate transaminase 1	8	7
11	Lactate dehydrogenase A	9	6
12	Lactate dehydrogenase B/peptidase B	8	7
13	Esterase D	11	4
14	Nucleoside phosphorylase	8	7
15	Mannose phosphate isomerase/pyruvate kinase M2	9	6
16	Adenine phosphoribosyltransferase	9	6
17	Galactokinase	5	10
18	Peptidase A	7	8
19	Glucose phosphate isomerase	8	7
20	Adenosine deaminase 1	10	5
21	Superoxide dismutase 1	8	8
22	Aconitase 2	9	6
X	Glucose 6-phosphate dehydrogenase	6	9

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