Assignment of the gene for the β subunit of thyroid-stimulating hormone to the short arm of human chromosome 1

(glycoprotein hormone/nerve growth factor/gene mapping)

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ABSTRACT The chromosomal locations of the genes for the β subunit of human thyroid-stimulating hormone (TSH) and the glycoprotein hormone α subunit have been determined by restriction enzyme analysis of DNA extracted from rodent-human somatic cell hybrids. Human chorionic gonadotropin (CG) α -subunit cDNA and a cloned 0.9-kilobase (kb) fragment of the human TSH β -subunit gene were used as hybridization probes in the analysis of Southern blots of DNA extracted from rodent-human hybrid clones. Analysis of the segregation of 5- and 10-kb *EcoRI* fragments hybridizing to CG α -subunit cDNA confirmed the previous assignment of this gene to chromosome 6. Analysis of the patterns of segregation of a 2.3-kb *EcoRI* fragment containing human TSH β -subunit sequences permitted the assignment of the TSH β -subunit gene to human chromosome 1. The subregional assignment of TSH β subunit to chromosome 1p22 was made possible by the additional analysis of a set of hybrids containing partially overlapping segments of this chromosome. Human TSH β subunit is not syntenic with genes encoding the β subunits of CG, luteinizing hormone, or follicle-stimulating hormone and is assigned to a conserved linkage group that also contains the structural genes for the β subunit of nerve growth factor (*NGFB*) and the proto-oncogene *N-ras* (*NRAS*).

Thyroid-stimulating hormone (TSH) controls the synthesis of the biologically active thyroid hormones (1). It is a member of the family of glycoprotein hormones that also includes placental chorionic gonadotropin (CG) and the pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Each of these four hormones is composed of two dissimilar noncovalently bound glycosylated subunits. The α subunit is identical in each of the glycoprotein hormones, but the β subunits are different and confer immunological and biological specificity to the complete hormone (2).

There appears to be only one α -subunit gene in all species studied to date, whereas there are multiple CG β -subunit genes in primates that have probably arisen by duplication of a single LH β -subunit gene (3–9). Although we had initially thought that there was more than one TSH β -subunit gene in the mouse, we now believe there is only a single TSH β -subunit gene in mice and probably also in humans (10, 11). We have previously shown that the genes for the α and β subunits of mouse TSH are located on different chromosomes, with the α subunit mapping to mouse chromosome 4 and the β subunit mapping to mouse chromosome 3 (12). The mouse LH β subunit has been assigned to mouse chromosome 7 (12, 13).

To map the human TSH β -subunit gene and to compare the chromosomal assignments of the genes for the glycoprotein hormone α subunit, LH β subunit, and TSH β subunit in humans and mice, we have used a human TSH β -subunit gene fragment as a probe in the analysis of Southern blots of DNA extracted from rodent-human somatic cell hybrids.

MATERIALS AND METHODS

Hybridization Probes. TSH β -subunit gene fragments were isolated from a human genomic library by using mouse and bovine TSH β -subunit cDNAs as probes (14, 15). The human genomic library was provided by A. Bank (Columbia University, New York), and the bovine TSH β -subunit cDNA was the gift of R. A. Maurer (University of Iowa, Iowa City). Nucleotide sequencing and comparison of the deduced amino acid sequence with the known amino acid sequence for human TSH β -subunit demonstrates that a 0.9-kilobase (kb) *EcoRI/BamHI* human TSH β -subunit gene fragment contains an exon coding for 34 amino acids of the amino terminus of human TSH β subunit plus a 20-amino acid apparent signal peptide (16). Human CG α -subunit cDNA was kindly provided by J. Fiddes.

Chromosomal Content of Somatic-Cell Hybrid Panels. The chromosomal localization of human TSH β subunit was determined by hybridization of the cloned human TSH β -subunit gene fragment to Southern blots containing DNA extracted from two independent panels of somatic-cell hybrids. The derivation and characterization of the panel of hybrids maintained in the Human Cancer Immunology Laboratory at Memorial Sloan-Kettering Cancer Center have been described (17–21). The NSK and NBE mouse-human hybrid clones are the result of the fusion of SK-N-BE(2) and BE(2)-C [a subclone of SK-N-BE(2)] human neuroblastoma cells with mouse N4TG-1 and NS20TG11E cells, respectively. The LC-1.3/35 hybrid was derived from the fusion of SK-MEL-131 human melanoma cells with mouse L cells, and CC4/16 and CE-25/1 hybrids from the fusion of SK-MEL-28 and SK-MEL-131 with Chinese hamster YH21 cells. Hybrids A9/1492 and A9/GM10 were derived from the fusion of normal human fibroblasts with mouse A9 cells. The presence of human chromosomes in these hybrids was determined by a combination of trypsin Giemsa banding, isozyme analysis, and serological analysis of chromosome-specific cell-surface antigens defined by monoclonal antibodies (17–21). The cell-surface markers included the 140-kDa glycoprotein determined by the locus *MSK1*, which has been assigned to chromosome band 1p22 (21).

A second panel of 15 rodent-human hybrid clones was analyzed at Baylor College of Medicine to provide an

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Abbreviations: TSH, thyroid-stimulating hormone; CG, chorionic gonadotropin; LH, luteinizing hormone; FSH, follicle-stimulating hormone; kb, kilobase(s).

independent confirmation of the assignment of the TSH β -subunit locus. This panel consists of 10 Chinese hamster-human hybrids and 5 mouse hepatoma-human lymphoblastoid hybrids (22, 23). The human chromosomal complement of these hybrids was determined by Giemsa 11 staining, trypsin Giemsa banding, isozyme analysis, and by Southern blot analysis of the chromosome-specific argininosuccinate synthetase pseudogenes (24).

Restriction Fragment Analysis of the Somatic-Cell Hybrids. Ten micrograms of DNA was digested to completion with *EcoRI* restriction enzyme (5 units/ μ g) (Boehringer-Mannheim and New England Biolabs) and separated in 0.8% agarose gels. The DNA was transferred to Nytran filters (Schleicher and Schuell) by the method of Southern (25). Filters were hybridized with recombinant plasmid DNA containing a 0.9-kb *EcoRI/BamHI* fragment of the human TSH β -subunit gene or human CG α -subunit cDNA labeled to an activity of $>1 \times 10^8$ cpm/ μ g (26). The filters were hybridized overnight at 42°C in the presence of 50% formamide. The filters were washed twice at room temperature in $2 \times$ SSC ($1 \times$ SSC = 0.15 M NaCl/0.015 M Na citrate)/0.1% NaDodSO₄ for up to 2 hr and three times in $0.1 \times$ SSC/0.1% NaDodSO₄ for 30 min each. The final two washes were done at 65°C. Filters were stripped for rehybridization by boiling in $0.01 \times$ SSPE ($1 \times$ SSPE = 180 mM NaCl/10 mM NaPO₄, pH 7.7/1 mM EDTA) and 1% NaDodSO₄ for 20 min.

The presence of *NGFB* and *NRAS* sequences in these hybrid clones was determined by hybridization with nick-translated N8C6 (27) and pNP-1 (28) plasmid DNA to the stripped filters containing *EcoRI*-digested hybrid-cell DNA.

RESULTS

Chromosomal Assignment of TSH β Subunit. A single 2.3-kb *EcoRI* fragment containing human TSH β -subunit sequences was detected in the human control DNA samples (Fig. 1). Hybridization with an 8.3-kb fragment in mouse and a 9.4-kb fragment in Chinese hamster control samples was detected in blots washed at lower stringency. The 2.3-kb human *EcoRI* fragment was detected in 22 of 35 hybrid clones (Table 1). Analysis of the hybrid panel demonstrated concordant segregation of this fragment with chromosome 1. All other chromosomes were excluded as the site for the TSH β -subunit gene by at least 11 discordant hybrids. Analysis of two highly reduced human fibroblast-derived hybrid clones, which only contain human genetic material derived from a segment of chromosome 1 (hybrid A9/1492/2) and an intact chromosome 1 and a segment of chromosome 10 (hybrid A9/GM10/19c), provides particularly strong evidence for the assignment of TSH β subunit to chromosome 1 since they are both positive for the 2.3-kb *EcoRI* fragment.

Subregional Assignment of TSH β Subunit. The subregional assignment of TSH β -subunit locus was undertaken on a panel of hybrids containing different segments of human chromosome 1 in the absence of the intact homolog (Fig. 2). The human parental cells for the NSK and NBE hybrid series contain a normal chromosome 1 and a t(1;?)(p22;?) marker

Table 1. Discordancy analysis of the combined panel of 35 rodent-human hybrid clones

Chromosome	TSH β subunit/ chromosome				Total	
	Concordant		Discordant		Concordant	Discordant
	+/+	-/-	+/+	-/-		
1	22	13	0	0	35	0
2	3	7	18	6	10	24
3	4	7	16	6	11	22
4	7	6	13	7	13	20
5	5	10	16	4	15	20
6	9	8	12	5	17	17
7	6	7	15	6	13	21
8	5	11	17	2	16	19
9	5	11	17	2	16	19
10	11	10	8	3	21	11
11	10	6	5	6	16	11
12	9	10	12	3	19	15
13	3	10	19	3	13	22
14	15	5	7	8	20	15
15	13	8	8	5	21	13
16	4	8	17	5	12	22
17	2	10	18	2	12	20
18	2	9	20	4	11	24
19	8	11	10	2	19	12
20	14	9	8	4	23	12
21	14	6	6	7	20	13
22	4	9	4	13	13	17
X	17	2	5	11	19	16

The human chromosomal complement of each hybrid was compared with the presence of 2.3-kb *EcoRI* restriction fragments containing human TSH β -subunit sequences. Concordant segregation of this fragment containing human TSH β -subunit sequences was observed only for chromosome 1. All other chromosomes were excluded as the site of the TSH β -subunit gene by discordancies in at least 11 hybrids. Hybrid clones containing rearranged chromosomes that did not involve human chromosome 1 and hybrid clones containing a chromosome in $<25\%$ of cells are excluded from the analysis of discordancy for those particular chromosomes. Consequently, the sum of concordant and discordant hybrids does not always equal 35.

chromosome (29). The marker chromosome has been retained in the absence of the normal homolog in eight of the NSK and NBE hybrids (17–21). Analysis of the presence of the 2.3-kb *EcoRI* fragment in hybrids containing this marker chromosome and three other hybrids containing different segments of human chromosome 1 shows that TSH β subunit is excluded from the region 1cen-qter by hybrid CC4/16, and that the smallest region of overlap on the three hybrids containing different regions of the short arm is band 1p22 (Fig. 2). TSH β subunit cosegregates with *NGFB*, *NRAS*, and *MSK1*, which have all previously been assigned to the same chromosome band.

Chromosomal Assignment of the Glycoprotein Hormone α Subunit. The chromosomal assignment of the glycoprotein

FIG. 1. Hybridization of ³²P-labeled pTSHB (0.9-kb gene) probe to Nytran filters carrying human and mouse parental cell and representative hybrid cell DNA samples. Human parental cell line SK-N-BE(2) (lane 1), mouse parental cell line N4TG-1 (lane 2), and hybrid cells NBE-E1, NBE-G1, NBE-H1, NBE-J2, NBE-K1, NBE-M1, NBE-N1, NSK1, NSK1s-1, NSK2, and NSK9 (lanes 3–13).

FIG. 2. Regional localization of the TSH β -subunit gene to human chromosome 1p22. Vertical bars represent the regions of chromosome 1 retained in individual hybrids in the absence of the normal homolog. The TSH β -subunit locus is excluded from the long arm of human chromosome 1 (1cen-qter) by hybrid CC4/16, and the smallest region of overlap among the three hybrids containing different regions of the short arm is band 1p22. TSH β subunit cosegregates with three loci (*NGFB*, *NRAS*, and *MSK1*), which have previously been assigned to chromosome band 1p22 (21, 37).

hormone α subunit was determined by hybridization of cloned human CG α -subunit cDNA to Southern blots containing DNA extracted from 20 rodent-human somatic cell hybrids. This panel consisted of the 16 NSK and NBE hybrids, and hybrids CC4/16, LC-1.3/35, A9/1492, and A9/GM10, which are described above.

The human α -subunit locus is polymorphic for two *Eco*RI fragments of 10 and 5 kb (3, 30). The human cell line SK-N-BE(2), which is the common human parental cell for the NSK and NBE hybrid series, is heterozygous at this locus and consequently has both the 5- and 10-kb *Eco*RI fragments. These two fragments segregate independently in the NSK and NBE hybrids. The 5-kb fragment is retained in five hybrids and the 10-kb fragment is retained in four hybrids. The discordancy analysis of the segregation of both *Eco*RI fragments containing α -subunit sequences with individual human chromosomes is presented in Table 2. Concordant segregation of the α subunit with human chromosome 6 was observed. All other chromosomes were excluded as the site of the glycoprotein hormone α -subunit gene by at least two discordant hybrids.

DISCUSSION

The β subunits of the glycoprotein hormones show variable degrees of amino acid sequence homology. CG β subunit and LH β subunit have an 82% amino acid sequence homology, while FSH β subunit and TSH β subunit have only 30–40% sequence homology with CG β subunit (2). Structural genes for CG β subunit and LH β subunit have both been mapped to human chromosome 19 (13, 31) and the FSH β -subunit gene has been mapped to chromosome 11 (32). There is a

Table 2. Discordancy analysis of the panel of 20 rodent-human hybrid clones

Chromosome	α subunit/chromosome				Total	
	Concordant		Discordant		Concordant	Discordant
	+/+	-/-	+/+	-/-		
1	4	1	1	3	5	4
2	2	9	7	1	11	8
3	0	11	7	0	11	7
4	4	8	4	2	12	6
5	2	10	7	1	12	8
6	8	11	0	0	19	0
7	4	11	4	0	15	4
8	2	11	7	0	13	7
9	2	10	7	1	12	8
10	5	6	3	3	11	6
11	5	5	1	1	10	2
12	6	8	3	2	14	5
13	1	11	8	0	12	8
14	9	6	0	5	15	5
15	8	4	1	6	12	7
16	3	10	5	1	13	6
17	1	10	6	0	11	6
18	0	10	9	1	10	10
19	3	6	4	3	9	7
20	7	6	2	5	13	7
21	6	5	1	6	11	7
22	1	10	7	1	11	8
X	6	4	3	7	10	10

The human chromosomal complement of each hybrid was compared with the presence of either the 5-kb or the 10-kb *Eco*RI restriction fragment containing human glycoprotein hormone α -subunit sequences. Concordant segregation of these fragments containing human α -subunit sequences was observed only for chromosome 6. All other chromosomes were excluded as the site of the α -subunit gene by discordancies in at least 2 hybrids. Hybrid clones containing rearranged chromosomes that did not involve human chromosome 6 and hybrid clones containing a chromosome in <25% of cells are excluded from the analysis of discordancy for those particular chromosomes. Consequently, the sum of concordant and discordant hybrids does not always equal 20.

single LH β -subunit gene, while seven CG β -subunit genes or pseudogenes in tandem and inverted pairs have been identified (6–8). The assignment of TSH β subunit to human chromosome 1p22 demonstrates that this gene is not syntenic with the structural genes for the β subunits of the other glycoprotein hormones. This also appears to be the case in the mouse, where the structural genes for LH β subunit and TSH β subunit have been assigned to mouse chromosome 7 and mouse chromosome 3, respectively (12, 13).

The human glycoprotein hormone α -subunit gene has been assigned to two different human chromosomes. Naylor *et al.* (13) analyzed a large panel of rodent-human somatic cell hybrids with different human parental cells and have assigned the α -subunit gene to human chromosome 6q12–q21. However, in a study of hybrid clones derived from the human JEG-3 choriocarcinoma cell line, Bordelon-Riser *et al.* (33) determined that the presence of human chromosomes 10 and 18 was necessary for the expression of human CG. Further analysis of JEG-3 by *in situ* hybridization with labeled α -subunit cDNA and CG β -subunit cDNA demonstrated that the α -subunit gene is on chromosome 18 and the β -subunit gene is on chromosome 10 in this cell line (34, 35).

In our study, hybridization of 32 P-labeled cloned human α -subunit cDNA (36) with Southern blots containing *Eco*RI-digested hybrid clone DNA demonstrated that 5- and 10-kb fragments containing the α -subunit sequences segregated with human chromosome 6 (Table 2). All other chromosomes were excluded by at least 2 discordancies. These results

independently confirm the previous assignment by Naylor *et al.* (13) of the α -subunit gene to human chromosome 6 and also demonstrate that the human glycoprotein hormone α -subunit gene and the TSH β -subunit gene are on different chromosomes, as they are in the mouse.

A conserved linkage group containing three homologous loci on the proximal region of the short arm of human chromosome 1 and mouse chromosome 3 has been described. This linkage group contains *NGFB* and *NRAS* in band 1p22 and *AMY1* and *AMY2* in band 1p21 (37–41). The assignment of the TSH β -subunit gene to human chromosome 1p22 and to mouse chromosome 3 demonstrates that the structural gene for TSH β subunit is part of this conserved linkage group.

Human LH β subunit also forms part of a conserved linkage group including two other loci on human chromosome 19 and mouse chromosome 7 (38). These other loci include human peptidase D (*PEPD*) and glucose phosphate isomerase (*GPI*) loci, which are homologous to mouse peptidase 7 (*Pep-7*) and glucose phosphate isomerase (*Gpi*). Therefore, the duplication of the ancestral subunit gene and the separation of the ancestral TSH β -, FSH β -, and LH β -subunit genes on different chromosomes occurred before the evolutionary divergence of mice and humans. The absence of CG in rodents (9, 42, 43) and the relative homology of human CG β subunit and LH β subunit indicate that the evolution of the LH β /CG β -subunit gene cluster is a relatively recent event occurring in the primate lineage.

Thyroid hormones decrease the biosynthesis of TSH, levels of TSH mRNAs, and TSH gene transcription (44–48). The regulation of TSH biosynthesis by thyroid hormones appears to be predominantly controlled by the suppression of the synthesis of the TSH β subunit (47, 48). Thyroid hormones have also been shown to regulate the synthesis of nerve growth factor, and it has been suggested that the influence of thyroid hormones on central nervous system development is mediated by nerve growth factor (49–51). The assignment of the gene encoding the human TSH β subunit to a conserved linkage group in chromosome 1p22, which also contains the gene for nerve growth factor β subunit, suggests a possible genetic, as well as functional, relationship between these two proteins. Nevertheless, more detailed genetic analyses will be needed to determine the exact proximity of the TSH β - and nerve growth factor β -subunit genes and the relative gene order of these two loci and the proto-oncogene *NRAS*, which is also located in chromosome band 1p22.

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