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STRUCTURE AND CHROMOSOME ASSIGNMENT OF HUMAN S100 α AND β SUBUNIT GENES

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The human S-100 α and β subunit genes were isolated from human genomic DNA library. The restriction endonuclease mapping and DNA sequencing analysis revealed that both subunit genes consisted of three exons and two introns. Two Ca^{2+}-binding domains were independently encoded by exon 2 and exon 3. Spot-blot hybridization analysis with flow-sorted chromosomes showed that human α and β subunit genes were assigned to chromosome 1 and chromosome 21, respectively.

S-100 protein was initially found as a brain specific protein (1). Isobe and Okuyama described that this protein was composed of two subunits: α and β which associate into αα ($S100a_0$), αβ (S100a) and ββ (S100b) (2, 3). Further they demonstrated the structural relation between S-100 protein and Ca^{2+}-binding proteins of the EF-hand type.

Although the primary amino acid sequences of α and β subunits are considerably similar, these subunits are differently distributed in mammalian brain. In human brain β subunit is exclusively localized in astrocytes in contrast to that α subunit is present in neurons (4). In rat brain α subunit is not present (5). These investigations suggest that there exists differential expression mechanism of each subunit gene in mammalian brain.

mRNA level of β subunit in rat brain increases rapidly after birth (6), suggesting that the formation of neuron network is associated with neurite extension activity of β subunit (7). Recently human β subunit gene has been assigned to chromosome 21 using segregating human-mouse hybrid cells and is believed to be involved in Down's syndrome through an excess of the gene

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products resulting from trisomy of the gene (8). These investigations suggest that ß subunit gene plays an important role in development and maturation of mammalian brain. In contrast, the function of α subunit in the central nervous system is unclear.

In order to study the molecular mechanism of gene expression of α and ß subunit in mammalian brain, we attempted cloning and chromosome assignment of human α and ß subunit genes.

METHODS

High molecular weight DNA was prepared from human placenta as described by Blin and Stafford (9). Genomic DNA was analyzed by Southern blot hybridization with human α and ß subunit cDNAs which we cloned previously. EMBL 3 human leucocyte genomic DNA library purchased from Clontech Laboratory was screened with the coding region of human α or ß subunit cDNA. Five positive clones were obtained using ß subunit cDNA as a probe. In contrast, no positive clones were identified using α subunit cDNA as a probe. Then, genomic DNA library was constructed as follows: Human genomic DNA was digested with EcoRI and preparatively electrophoresed on 0.8% agarose gel. About 6 kbp fragment corresponding to single hybridization band in Southern blot analysis using the entire length of human α subunit cDNA as a probe was inserted into the EcoRI site of λgt10 DNA and packaged in vitro. The 6 kbp genomic DNA library was screened with human α subunit cDNA. The restriction map of the cloned DNA was determined by restriction enzyme analysis and Southern blot analysis. The nucleotide sequences were determined by the dideoxy method (10).

Human metaphase chromosomes were prepared from human B-lymphoblast line GM00130B as described before (11). Chromosomes stained with propidium iodide were excited under 488 nm light, while those stained with Hoechst 33258 were excited with 333-364 nm light, both generated with an argon-ion laser. Sorting of chromosomes by a FACS440 sorter (Becton-Dickinson) and spot-blot hybridization were performed as described before (11). Assignment of chromosomes in each fraction was carried out with chromosome-specific DNA probes. Filter disks were hybridized with the ^{32}P-labeled human α and ß subunit cDNA probes as described previously (11).

RESULTS AND DISCUSSION

Human genomic DNA library was screened with human α and ß subunit cDNA probes which we cloned previously. Genomic clones of 6 kbp α subunit and those of 14 kbp ß subunit were isolated and the restriction map of each subunit gene was determined (Fig. 1a). Both subunit genes consisted of three exons and two introns (Figs. 1b and 2). The exon 1 consisted of only the 5' noncoding region (α and ß). The exon 2 encoded 13 nucleotides (α) or 1 nucleotide (ß) of the 5' noncoding region and 141 nucleotides (α) or 138 nucleotides (ß) of the coding region. The exon 3 encoded 141 nucleotides (α) or 138 nucleotides (ß) of the coding region and the entire 3' noncoding

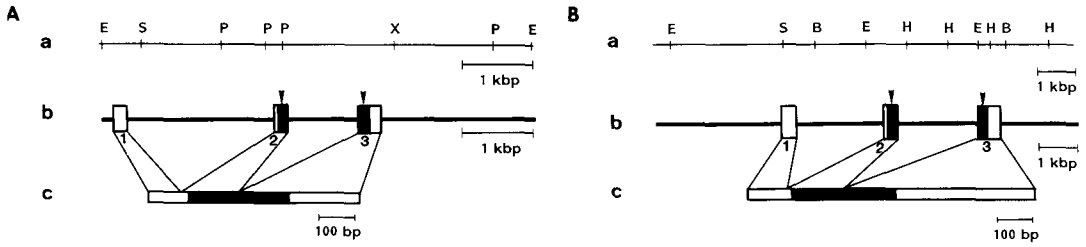

Fig. 1. Schematic presentation of human α subunit gene (A) and human β subunit gene (B). (a) Restriction map of genomic clone. B, BamHI; E, EcoRI; H, HindIII; P, PstI; S, SmaI; X, XbaI. (b) Diagram of the gene. Exons, indicated by the boxes, are numbered. The solid boxes indicate the coding region and the open boxes indicate the noncoding region. The arrowheads identify the Ca^{2+}-binding domains. (c) Structure of the cloned cDNA.

region (α and β). The lengths of introns were as follows: intron 1, 2.0 kbp (α) or 2.5 kbp (β); intron 2, 0.9 kbp (α) or 2.3 kbp (β). All exon-intron junction sequences conformed to the GT-AG rule. The exons of each subunit gene were colinear with each subunit cDNA sequence. Atypical polyadenylation

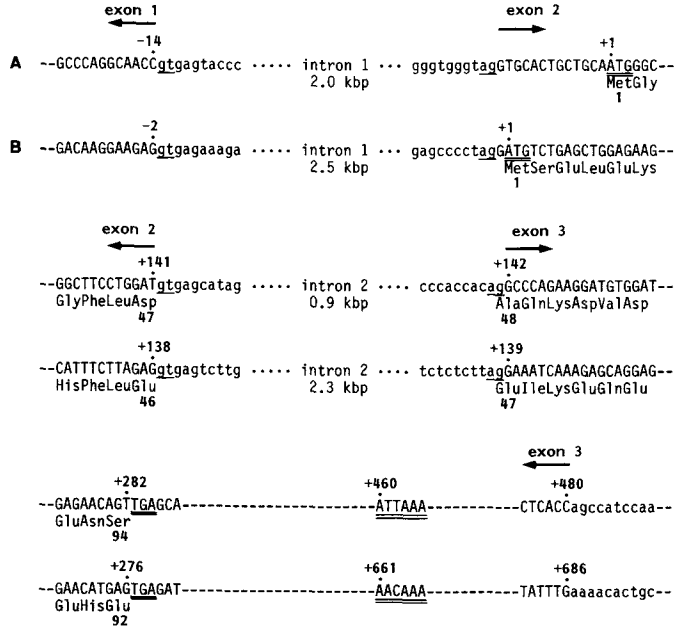

Fig. 2. Comparison of human α subunit gene (A) and human β subunit gene (B). Exons are denoted in uppercase letters, while introns are in lowercase letters. The nucleotides in the exons are numbered above the lines beginning with the first nucleotide of the initiation methionine codon. The nucleotides on the 5' side of nucleotide 1 are indicated as negative numbers. The nucleotides in the introns, the approximate lengths of which are shown below the lines, are not numbered. Splice junction sequences are underlined. Translation initiation codon, stop codon, and the polyadenylation signal are double-underlined. The amino acid sequences are shown below the genomic sequences and the amino acids are numbered below the lines beginning with the initial methionine.

signal, AACAAA, which was present 21 nucleotides upstream from the poly(A) tail of human ß subunit cDNA, was observed also in ß subunit gene.

Comparison of the nucleotide sequences of the 5' flanking region of human α subunit gene and human ß subunit gene is shown in Fig. 3. The sequences of human α and ß subunit genes showed moderate similarity. In addition, human ß subunit gene and rat ß subunit gene (12) shared remarkably high homology in the nucleotide sequences of the 5' flanking region (data not shown).

The coding region of human α and ß subunit genes was divided equally by intron 2 (Figs. 1b and 2). The same manner was found in rat ß subunit gene (12) (data not shown). S-100 α and ß subunits contain two Ca^{2+}-binding domains known as EF hand (2, 3). In both subunits two Ca^{2+}-binding domains

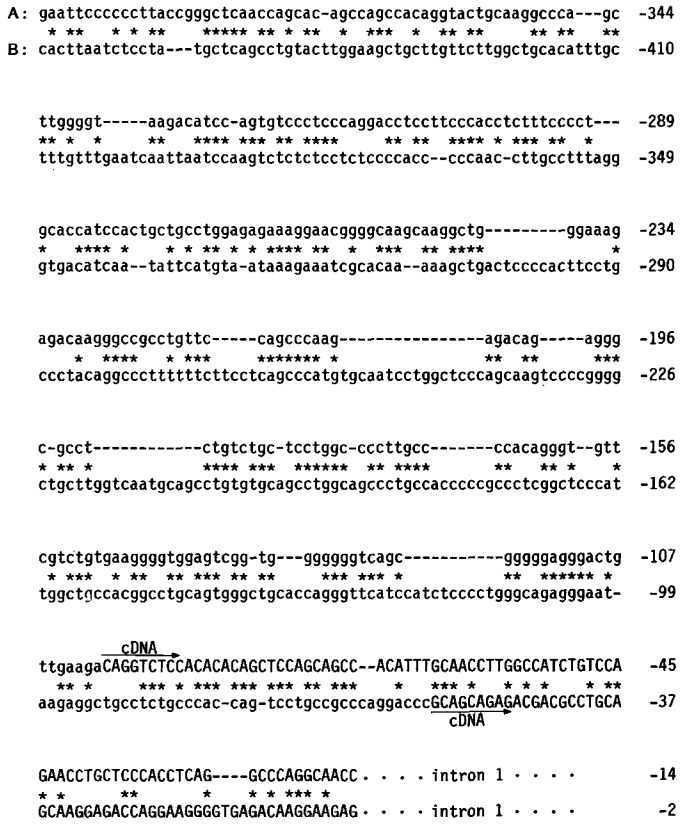

Fig. 3. Comparison of the nucleotide sequences of the 5' flanking region of human α subunit gene (A) and human ß subunit gene (B). The sequences of cDNA are denoted in uppercase letters, while the upstream flanking sequences of gene are in lowercase letters. The last nucleotide in the line is numbered beginning with the first nucleotide of the initiation methionine codon. The nucleotides in intron 1 are not numbered.

were independently encoded by exon 2 and exon 3 (Fig. 1b). These findings support the hypothesis that EF-hand type proteins may arise evolutionally from an ancestral peptide with one Ca^{2+}-binding domain by several steps of gene duplications and deletions (13).

Southern blot analysis of human genomic DNA was performed using human α or ß subunit cDNA (Fig. 4). The pattern of hybridization bands consisted with the restriction map of each subunit gene, suggesting that there exists only one copy of each subunit gene per haploid genome.

Spot-blot hybridization using flow-sorted chromosomes was performed. Sorting of chromosomes from the human B-lymphoblast line GM00130B stained with propidium iodide gave 18 groups (a_1-q) (Fig. 5A). α subunit cDNA hybridized to the a_1 fraction (Fig. 5B) and ß subunit hybridized to the q fraction (Fig. 5C). Therefore, α and ß subunit genes could be assigned to human chromosome 1 and 21, respectively. The same results were obtained using the flow-sorted chromosomes stained with Hoechst 33258 (data not shown). These results confirmed the previous chromosome assignment of human ß subunit gene using segregating human-mouse hybrid cells (8) and provided new evidence for α subunit gene localization. Thus, α and ß subunits did not form gene family

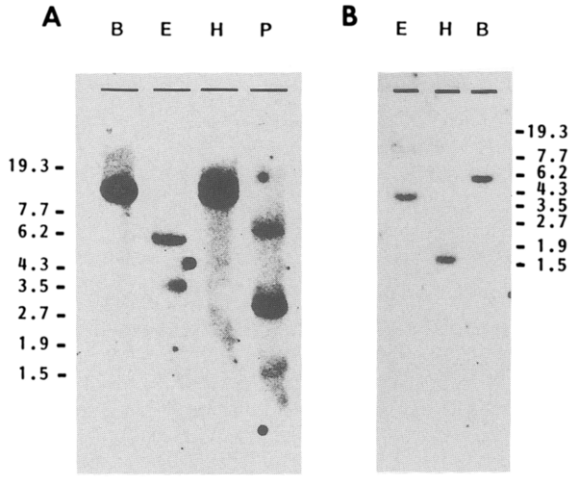

Fig. 4. Southern blot analysis. Human genomic DNA was digested with several endonucleases. B, BamHI; E, EcoRI; H, HindIII; P, PstI. Hybridization was carried out with the entire length of human α subunit cDNA (A) and with the 3' noncoding region of human ß subunit cDNA (B). The size markers are indicated in kbp.

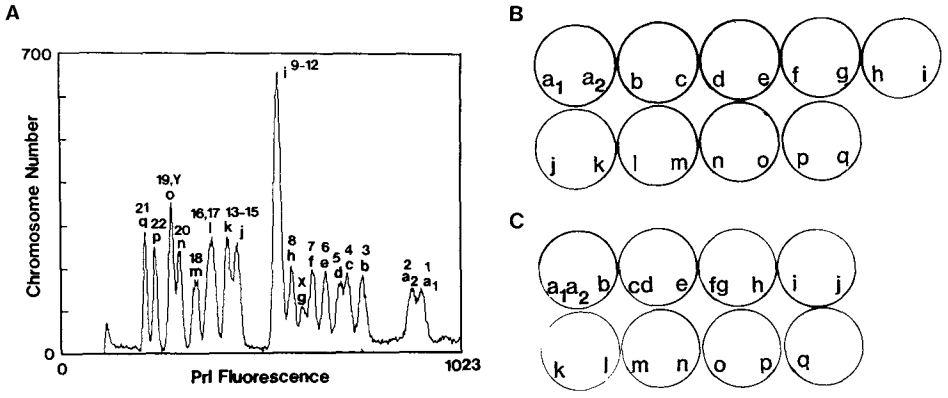

Fig. 5. Localization of the human α and β subunit genes on the flow-sorted chromosomes from the human B-lymphoblast line GM00130B. (A) Propidium iodide-stained flow-karyotype. (B) Hybridization of the entire length of human α subunit cDNA probe to spots containing sorted chromosomes. (C) Hybridization of the 3' noncoding region of human β subunit cDNA probe to spots containing sorted chromosomes. In this case, fractions a_1 and a_2 were collected as a mixture.

locus on the same chromosome. Similar observations have been made for cases of isozymes such as enolase (14, 15) or aldolase (16-18). The genes for their subunits, displaying structural similarity, are located on separate chromosomes without forming gene family locus.

Structure and chromosome assignment of human S100 α and β subunit genes are described in this study. Further experiments are necessary to clarify the role of S-100 protein in cellular function and the molecular mechanism that controls the expression of α and β subunit genes during development and maturation of mammalian brain.

During the preparation of this manuscript the cloning of human S-100 β subunit gene has been reported (19).

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