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MOLECULAR CLONING OF cDNA ENCODING HUMAN DREBRIN E AND CHROMOSOMAL MAPPING OF ITS GENE+

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Summary: Drebrins are novel actin-binding proteins in the brain which are developmentally regulated.Three isoforms: two embryonic types (E1 and E2) and an adult type (A) are generated by alternative RNA splicing from a single drebrin gene in the chicken brain. A full length cDNA clone of human drebrin E has been isolated from a cDNA library of human fetus brain. The clone is 2596 base pairs in length and contains an open reading frame of 1947 nucleotides encoding a protein of 649 amino acids The deduced amino acid sequence, except for the internal 138-nucleotide sequence (ins2), exhibits 88% homology with rat drebrin A. Spot blot hybridization using flow-sorted human chromosomes provides evidence that the gene encoding human drebrin protein locates on human chromosome 5.

Actin-binding proteins, drebrins, have been demonstrated to be developmentally regulated in the process of neuronal growth (1, 2, 3). Three isoforms: two embryonic types(E1 and E2) and an adult type(A) are generated by alternative RNA splicing from a single drebrin gene, in the chicken brain (4, 5, 6). The expression of drebrins in the chick optic tectum has been surveyed in detail by two-dimensional gel electrophoresis (7). Drebrin E1 and E2 appear transiently at the developmental stage corresponding to the beginning of the neuronal differentiation and outgrowth of the neural processes, respectively. On the other hand, drebrin A appears in parallel with further maturation of the neurons and remains in the adult brain (8). The significance of drebrins in the cell morphogenesis was first demonstrated by transfection of fibroblasts with the rat drebrin A expression plasmid (3). Transient expression of drebrin A induces the formation of highly branched neurite-like cell processes in these non-neuronal cells.

+ The sequence data in this paper have been submitted to the DDBJ, EMBL, GenBank DNA databases under the accession number D17530.

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Drebrin A is concentrated on the inner face of the cell membrane. Furthermore, drebrins are colocalized with actin filaments in the neurons and neuroblastoma cells (Asada et al., submitted).

In this study, we isolated a full length cDNA clone of drebrin E by screening a cDNA library constructed from fetal human brain and analyzed the structure. And we have mapped the human drebrin gene to chromosome 5 by spot blot hybridization using flow-sorted human chromosomes.

Materials and Methods

Isolation of cDNA and determination of the cDNA sequences: A cDNA library of 21-week-old human fetal brain tissues constructed with lambda gt11(CLONTEC Lab. USA) was screened with rat drebrin A cDNA as a probe. Recombinant phages were plated with E. coli Y1090 and transferred to a nylon membrane. The probe was labeled by a random primer method using [^{32}P]dATP and the Klenow fragment of DNA polymerase I (9). Filters were hybridized with ^{32}P-labeled probe overnight at 42°C in 5 x SSPE containing 50% formamide, 0.2% SDS, and 100 μg/ml salmon sperm DNA. They were then washed twice at 60°C in 2 x SSC containing 0.1% SDS and exposed to X-ray film. The human fetal brain cDNA library was screened again with the largest insert from positive clones serving as a probe. Positive clones were selected and EcoR I inserts were cloned into pUC 119 vector. The DNA sequence was determined by the dideoxy chain termination procedure using the T7 polymerase system (Pharmacia).

Spot blot hybridization using flow-sorted human chromosomes: Sorted chromosomes were from a human B-lymphoblastoide cell line, GM00130B. These cells showed an apparently normal karyotype. Preparation, staining, sorting, and hybridization of metaphase chromosomes with a DNA probe were previously described(10, 11, 12). Briefly, metaphase chromosomes were prepared by the polyamine/digitonin method, stained with either propidium iodide or Hoechst 33258, and sorted by a FACS440 cell sorter(Becton-Dickinson). Assignment of the chromosomes in sorted fractions was made according to Lebo et al.(13) and independently confirmed with chromosome-specific DNA probes. Fifty thousand chromosomes of each type were sorted as a small spot on nitrocellulose filter disks and treated for hybridization with the ^{32}P-labeled cDNA probe. Filters were washed with 0.5 x SSC containing 0.1% SDS at 63°C for 40 min and autoradiographed for 12 h.

Results and Discussion

Immunoblots have shown that drebrins are classified into three forms, E1, E2 and A, in the chicken (14). Molecular cloning of three types of chicken drebrin cDNAs have revealed that the heterogeneity of chicken drebrins is obtained by insertion or deletion of the two sequences, ins1 and ins2 (138-bp sequence), in the 5' direction immediately upstream from ins1 (4). Drebrin E1 mRNA excludes both ins1 and 2, drebrin E2 mRNA includes ins1, but not ins2, and drebrin A mRNA includes both these insertion sequences. Two forms of immunoreactive molecules are also present in the brains of mammals, including rat (2) and cat (15). In mammals, rat drebrin A cDNA has been isolated and characterized (3).Since the sequence of ins2 is well conserved between chicken and rat, it is proposed that this insertion also results in the heterogeneity in rat drebrins.

Approximately 5 x 10^5 recombinant phages were screened with the ^{32}P-labeled rat drebrin A cDNA as a probe, and 13 positive clones were isolated. The two clones, gDbh9 and gDbh13, containing larger inserts were chosen for further analysis. Furthermore, 1.5 x 10^6 recombinant phages were screened with the largest insert from positive clones, gDbh13 as a probe and two independent clones, gDbh13-2, gDbh13-3, were isolated. Four inserts had identical restriction maps and are apparently derived from the same mRNA. To further characterize these clones, the longest clone, gDbh13, was chosen for determining the nucleotide sequence; the result is presented in Fig.1 There is a long open reading frame starting from the initiating codon ATG at nucleotide position 98-100 and ending at the termination codon TAG at nucleotide position 2045-2047. The open reading frame encodes a protein consisting of 649

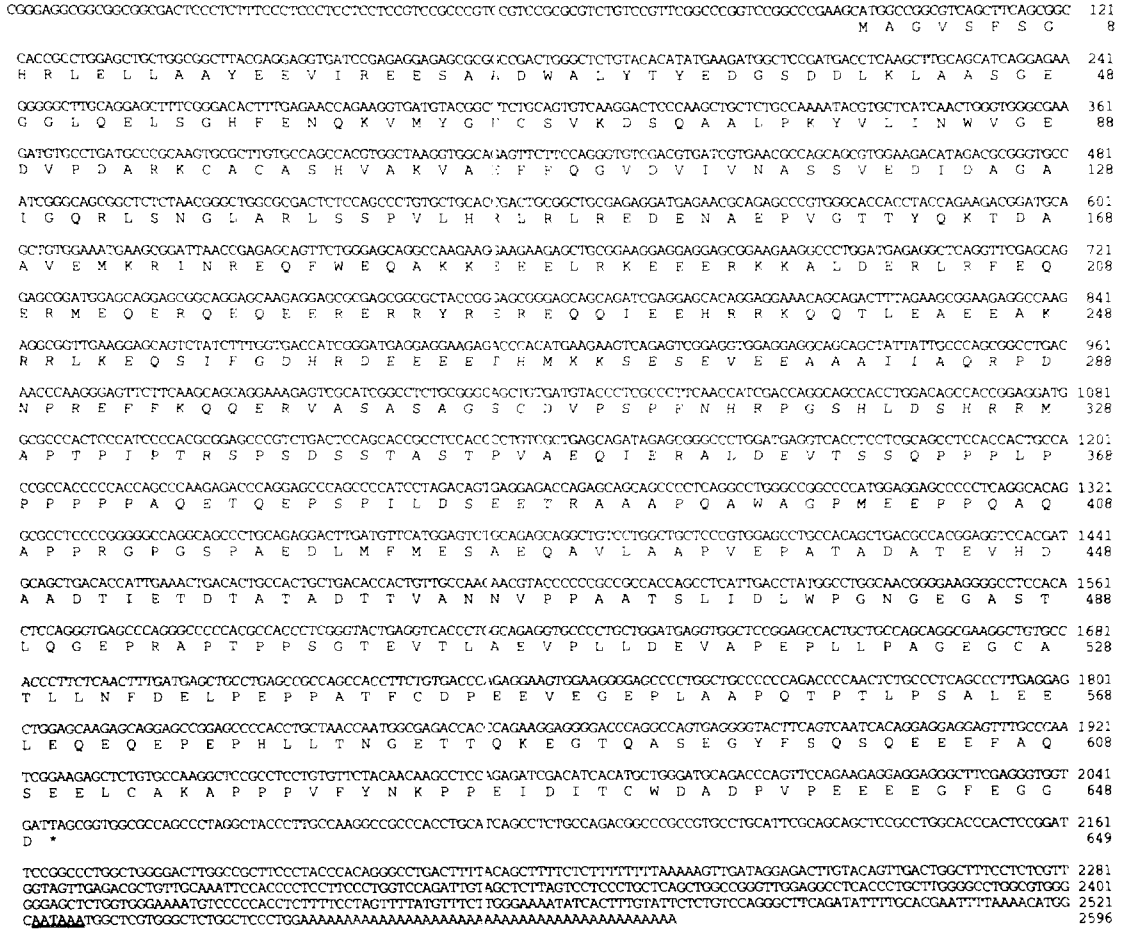

Fig. 1. Nucleotide sequence of human drebrin E cDNA, gDbh13, and its deduced amino acid sequence. The amino acid sequence is written in one-letter code below the nucleotide sequence. Polyadenylation signal is underlined.

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Rat      961  CCTCTGGTGGCAGCTGTGACGCACCCTCGCCCTTCAACCACCGACCAGGTCGTCCGTACT
              ******  ********** * ******************** *******
Human   1005  CCTCTGCGGGCAGCTGTGATGTACCCTCGCCCTTCAACCATCGACCAG----------

Rat     1021  GCCCTTTCATAAAGGCATCGGACAGTGGGCCTTCCTCCTCCTCCTCTTCCTCCTCTTCCC
Human         ----- ------ ---------- ---------- ---------- ----------

Rat     1081  CTCCACGGACTCCCTTTCCCTATATCACCTGCCACCGCACCCCAAACCTCTCTTCCTCCC
Human         ---------- ---- ---- ---------- ---------- ---------- -------

Rat     1141  TCCCATGCAGTCACCTGGACAGCCACCGGAGGATGGCGCCCACTCCCATTCCCACCCGGA
                    **** ********************************************* ****
Human   1053  -----GCAGCCACCTGGACAGCCACCGGAGGATGGCGCCCACTCCCATCCCCACGCGGA
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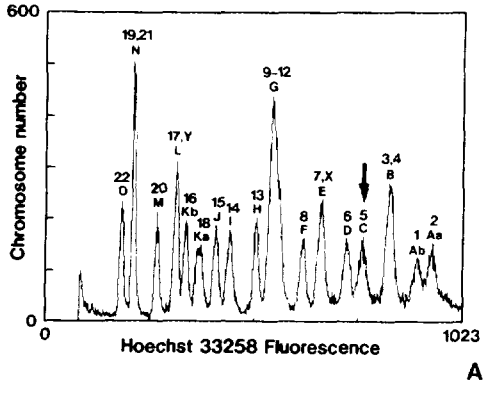
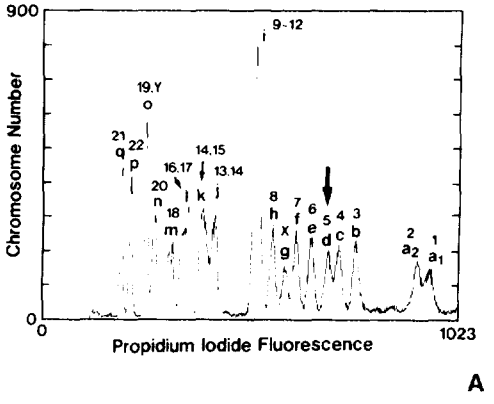
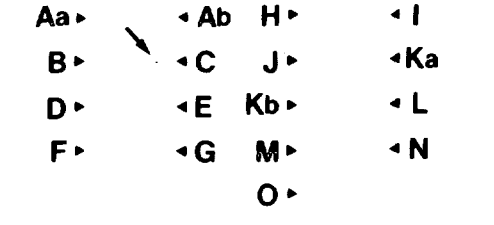
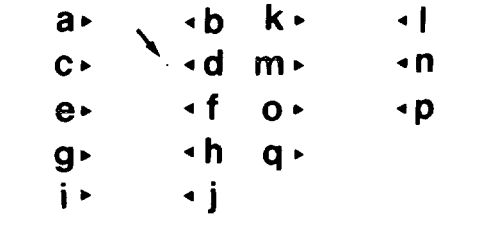
Fig. 2. Comparison of nucleotide sequence of rat drebrin A and human drebrin E. Asterisks represent the same nucleotide residues. Shaded rectangles indicate the internal sequence (138 bp), designated ins 2.









③ B ④ B

Fig. 3. (A) A typical flow karyotype of GM00130B chromosomes. Metaphase chromosomes were stained with 2 μg/ml Hoechst 33258. Numbers on each fraction represent the position of the corresponding chromosome types. (B) Chromosomal assignment of the drebrin gene. Fifty thousand chromosomes of each fraction were sorted directly onto nitrocellulose membrane disks and hybridized with human drebrin E cDNA.

Fig. 4. (A) A flow karyotype of GM00130B chromosomes. Metaphase chromosomes were stained with 35 μg/ml propidium iodide. Numbers on each fraction represent the position of the corresponding chromosome types. (B) Chromosomal assignment of the drebrin gene. Fifty thousand chromosomes of each fraction were sorted directly onto nitrocellulose membrane disks and hybridized with human drebrin E cDNA.

amino acids. Computer-aided sequence analysis revealed no overall homology with any other protein sequences in NBRF Protein Identification Resource Files. Figure 2 shows that the clone of gDbh13 does not include the internal sequence, ins2. The deduced amino acid sequences, except for ins2, exhibits 88% homology with rat drebrin A. According to these results, we name gDbh13 human drebrin E.

Spot blot hybridization of flow-sorted chromosomes was carried out to determine the chromosomal localization of the human drebrin E gene. GM00130B cell chromosomes stained with Hoechst 33258 were separated into 16 groups (A through O), consisting of one to four types of chromosomes (Fig. 3A). Fifty thousand chromosomes of each type were sorted and hybridized with [^{32}P]human drebrin E cDNA probe. As shown in Fig. 3B, only group C, containing chromosome 5, revealed a significant signal. Figure 4A shows that chromosomes stained with propidium iodide were separated into 17 groups (a through q). The same cDNA probe hybridized only with chromosomes in group D (Fig. 4B), containing chromosome 5. These results indicate the drebrin gene maps to human chromosome 5.

Acknowledgments

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