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Structure of the human gene for monoamine oxidase type A

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ABSTRACT

Monoamine oxidases, type A and type B, are principal enzymes for the degradation of biogenic amines, including catecholamines and serotonin. These isozymes have been implicated in neuropsychiatric disorders. Previously, cDNA clones for both MAO-A and MAO-B have been sequenced and the genes encoding them have been localized to human chromosome Xp11.23–Xp11.4. In this work, we isolated human genomic clones spanning almost all the *MAOA* gene from cosmid and phage libraries using a cDNA probe for MAO-A. Restriction mapping and sequencing show that the human *MAOA* gene extends over 70 kb and is composed of 15 exons. The exon structure of human *MAOA* is similar to that described by others for human *MAOB*. Exon 12 (bearing the codon for cysteine, which carries the covalently bound FAD cofactor) and exon 13 are highly conserved between human *MAOA* and *MAOB* genes (92% at the amino acid level). Earlier work revealed two species of MAO-A mRNA, 2.1 kb and 4.5–5.5 kb. We now report on further cDNA isolation and sequencing, which demonstrates that the longer message has an extension of 2.2 kb in the 3′ noncoding region. This extended region is contained entirely within exon 15. The two messages therefore appear to be generated by the use of two alternative polyadenylation sites. Results from the present work should facilitate the mutational analysis of functional domains of MAO-A and MAO-B. Knowledge of the gene structure will also help in evaluating the role of genetic variations in MAO-A in human disease through the use of genomic DNA, which is more accessible than the RNA, as a template for PCR-amplification and sequencing.

INTRODUCTION

Monoamine oxidases (MAO; monoamine: O_2 oxidoreductase; EC1.4.3.4.), type A and type B, are principal enzymes for the degradation of biogenic amines, (for review see 1,2,3). They catalyze the oxidative deamination of amine neurotransmitters such as dopamine, norepinephrine, epinephrine and serotonin, as well as dietary amines such as tryptamine and phenylethylamine. These enzymes are located in the outer membrane of mitochondria and are present in all types of cells, albeit at widely varying levels. Intensive biochemical and pharmacological studies of MAO have been stimulated from findings that inhibitors of the enzymes can be used as antidepressants (4). These studies have led to the characterization of two forms of the enzyme, MAO-A and MAO-B, which differ in specificity for substrates, sensitivity to inhibitors, tissue distribution, antigenic determinants, protein size and peptide maps (for review see 1, 2, 3). It is now clear from cDNA clones that these MAO isozymes are encoded by separate genes, which share about 70% overall homology in amino acid sequence (5–9).

Both human MAO genes have been mapped to the chromosome X in the p11.23–11.4 region (10–14). Absence of both genes has been described in atypical, male Norrie disease patients from four different families, who have a submicroscopic deletion in this region of the X chromosome (13, 15–18; Z.Chen and I.Craig, unpublished data; F.Collins and S.Antonarakis,

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manuscript submitted). It is conceivable that some features of their disease phenotype, such as microcephaly, mental retardation, seizures, and sleep disturbances, may result from the complete absence of MAO enzymatic activities in these patients throughout development and in later life. Other studies have shown that variant activities of platelet MAO-B are statistically associated with a number of neuropsychiatric diseases including affective disorders (19, 20), schizophrenia (21) and alcoholism (22, 23). A potential role of MAO in diseases of the nervous system is further suggested by findings that MAO-B can convert MPTP into a neurotoxin that causes neurodegeneration similar to Parkinson disease (24). In addition, deprenyl, an inhibitor of MAO-B, appears to be effective in slowing down the progression of Parkinson disease (25, 26). Allele association studies of MAO-A indicate that genetic variations at the structural locus are major determinants of activity levels in human skin fibroblasts (27). The structure of the human *MAOA* gene is reported here as a basis for studying the regulation of enzyme activity and its etiological role in human diseases.

MATERIAL AND METHODS

Isolation of genomic DNA and cDNA clones

Genomic clone A2 was isolated from a cosmid library prepared from a Sau IIIA partial digest of human genomic DNA (gift from Dr. Patricia Watkins, Integrated Genetics, Framingham, MA) established in the vector c2XB (28). A full length human cDNA clone for MAO-A, HM11 (6) was used as a probe, unless otherwise indicated. Genomic clone 5-5C (gift from Ms. Laurie Ozelius, Massachusetts General Hospital) was isolated from a cosmid library prepared from a Sau IIIA partial digest of genomic DNA from a homozygotic patient of Huntington disease (gift from Dr. James Gusella, prepared by Stratagene, La Jolla, CA) cloned in the vector pWE15 (29). Genomic clones 6.12, common, 4.15, 1.23 and 6.1 were isolated from an amplified human library prepared from an EcoRI partial digest of genomic DNA from a 4X female (gift of Dr. Dereck Blake, Oxford, U.K.) cloned in EMBL vectors (Promega). Genomic clone gMAO-A1 was isolated from a human lymphocyte lambda DASH genomic library (gift of Dr. Lily Hsu, Beckman Res. Inst., City of Hope, Duarte, CA, prepared by Stratagene) and probed with a 2.8 kb cDNA clone, pMAOA1, from a human placental cDNA library (prepared by Clontech). Two cDNA clones, CP221 and CP223, were isolated from a human placental cDNA library in lambda gt10 (Clontech Laboratories, Inc., Palo Alto, CA). Colony or plaque screening was done according to standard procedures (30).

Southern hybridization and restriction mapping

Procedures for blotting of DNA onto nylon filters, hybridization and autoradiography have been described previously (12). DNA probes were labeled with ^{32}P dCTP by random hexamer priming (31). The orientation of the MAO-A coding region relative to the vector was determined by probing with fragments from the 5′ and 3′ ends of HM11, a 0.15 kb AvaI fragment and a 0.52 kb AvaI fragment, respectively. Restriction mapping of clones 6.12, common, 4.15, 1.23 and 6.1 was done by single and double digestions with restriction enzymes, EcoRI, HindIII, SalI and BglII. Fragments were resolved by agarose gel electrophoresis, and the orientation was determined by probing with gel-purified fragments of SacI digested HM11 cDNA, which yielded three fragments of 528, 648 and 788 bp in the 5′ to 3′ direction. The sizes of introns 1, 3 and 5 were determined by hybridizing oligoprimers (Milligen-BioSearch/Cyclone DNA synthesizer) derived from exon 2, 4 and 5 to a Southern blot of DNA from a human MAO-A-bearing YAC (pYAC4 from Center for Genetics in Medicine, Washington Univ., St. Louis, MO) partially digested with rare cutting enzymes and resolved by pulse-field gel electrophoresis (Z.-Y.Chen and I.Craig, manuscript in preparation).

Analysis of DNA sequence

DNA sequences near intron-exon junctions were determined either by subcloning genomic fragments into appropriate vectors followed by sequencing; or by PCR-amplification of total genomic DNA and direct sequencing. These two approaches are briefly described as follows: (A) DNA fragments from restriction digestion were cloned into M13 (32), Bluescript KS (Stratagene, La Jolla, CA) pUC9 or pUC19 (33) vectors and sequenced by the chain-termination method (34) using modified T7 DNA polymerase (35; Sequenase Version 2.0 sequencing kit, United States Biochemical Co., Cleveland, OH) and [alpha-^{35}S]-dATP (36) with chain elongation on ice. Double stranded DNA sequencing was done by the alkaline-denaturation method (37). (B) Genomic DNA was amplified by polymerase chain reactions (38) using thermostable *Taq* DNA polymerase (GeneAmp kit, Perkin-Elmer Cetus, CA) according to manufacturer's instruction. Typically the reactions were done in 50 μl total volume in the presence of 200 pmoles of primers in 30 cycles (1 min at 94°C, 2 min at 55°C and 3 min at 72°C) with a final extension for 10 min. The amplified DNA fragments were separated by electrophoresis on a 2% low melting temperature agarose (SeaPlaque or NuSieve GTG agarose from FMC). Desired DNA bands were electroeluted onto DEAE membrane (Schleicher & Schuell), as described (39); or excised, melted and used subsequently for direct sequencing according to a simplified method (40) with minor modifications using Taq polymerase and [alpha-^{32}P]d-CTP, or Sequenase (41) with minor modifications and [alpha-^{35}S]dATP.

RESULTS

We have isolated genomic clones from three libraries (see Material and Methods). Clones 6.12, common, 4.15, 1.23 and 6.1 are from a phage library and cover most of the MAOA gene. All clones, together with those isolated from other libraries, including A2, gMAO-A1 and 5-5C were aligned to yield a region for MAOA covering about 70 kb. These clones were analyzed by Southern analysis of restriction digests using various fragments from the cDNA, HM11, as probes. Detailed alignment was done by superimposition of restriction sites for EcoRI, HindIII, BglII and SalI. A composite restriction map for these enzymes is shown in Figure 1. Exon 1 contains 5′-noncoding sequences corresponding to the cDNA clone hMAO A-7 (5) (Figure 2). The 5′ end of the first exon has not been defined precisely, but a potential TATA box appears 194 bp from the 5′ end of the published cDNA sequence, which may serve as a promoter element. A comparison between the two published cDNA sequences, HM11 (6) and hMAO A-7 (5), revealed that a 35 nucleotide segment in the 5′-noncoding region of HM11 (from nucleotide no. 8 to no. 42 in that reference) is an inverted repeat of a segment in hMAO A-7 (from nucleotide no. 31 to 65 in that reference). Since sequences from hMAO A-7 have also been

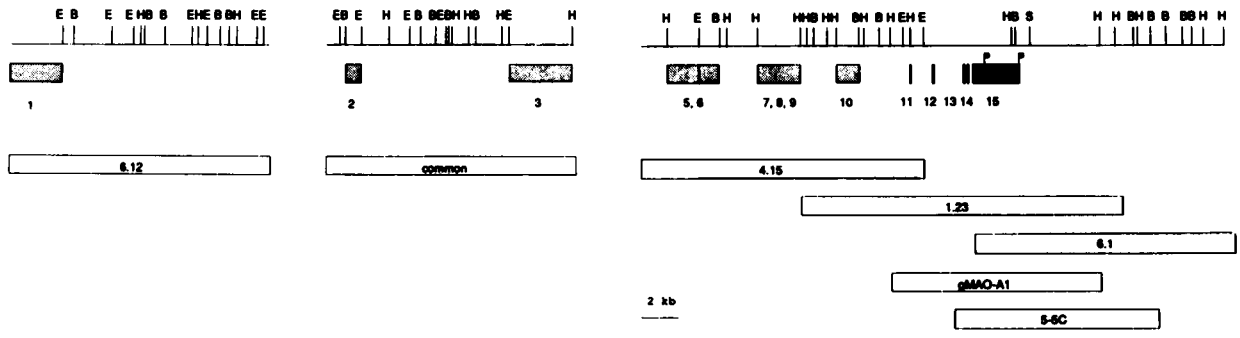


Figure 1. Structure of human *MAOA* gene. Recognition sites for restriction endonucleases HindIII, EcoRI, BglII and SalI are represented by letters H, E, B, and S, respectively. The solid horizontal bars beneath the restriction map indicate the position and extent of some exons, as determined by sequencing; the solid flags indicate polyadenylation signals. Open dotted bars indicate restriction fragments within which exons lie, as determined by hybridization of cDNA fragments to restriction digested genomic DNA clones. The open boxes below the exons show the genomic clones of *MAOA* used, with the names inside the bars.

found in genomic clones (Figure 2), it appears those in HM11 are a cloning artifact.

We have determined the exon structure of *MAOA* by two approaches. (A) Genomic clones were subcloned into sequencing vectors and sequenced. (B) Genomic DNA fragments were amplified using the polymerase chain reaction with appropriate primers from the cDNA sequence to generate DNA segments for direct sequencing. The latter approach was feasible only for segments containing exons and introns totalling less than 3 kb. The structure of the exons and nucleotide sequences near the intron/exon junctions are shown in figure 3. All exon-intron junctions showed appropriate consensus signals for splicing; GT at the 5′ end of introns and AG at the 3′ end. The gene contains 15 exons. All but exon 4 were analyzed in this study. The approximate size of the introns 1, 3 and 5 were determined by Southern blot hybridization to fragments generated by pulsed field gel electrophoresis of a YAC clone containing human MAOA (provided by Dr. Bernard Brownstein, Washington Univ.) digested with rare cutting enzymes (Z.-Y.Chen and I.Craig, manuscript in preparation). The size of other introns was evaluated by PCR amplification across them and/or by sequence analysis.

The cDNA clones from human placenta, CP223 and CP221, are 2.3 kb and 2.8 kb in length, respectively (6, 7). Partial sequencing was done to align these clones with HM11. The 5′ end of CP223 starts about 100 bp downstream from the 5′ end of HM11, and contains about 200 bp of additional sequence at the 3′ end. The 5′ end of CP221 starts about 700 bp upstream from the 3′ end of HM11, goes beyond the 3′ end of HM11 for an additional 2.1 kb. The combined sequences of these three cDNA clones covers 4.0 kb of the longer mRNA (7). The sequence AATAAA, which is a consensus signal for polyadenylation, is found 194 bases upstream from the 3′ end of CP221. A cDNA clone similar to CP221 has been obtained by Huang and co-workers (42). The 3′ non-coding sequences in CP221 are also found in genomic clones 5-5C and gMAO-A1. This 3′ end, including the other AATAAA polyadenylation signal found in HM11, plus all additional sequences of CP221 homologous to HM11 are contained within exon 15, which is 2.4 kb in length. Previously observed RNA species, 2.1 kb and 4.2 kb, for MAO-A from human placenta and liver (5,6), are similar in size to HM11 and to HM11-CP221 combined sequences, respectively, assuming that poly-A of tails about

TCTAAACCTA ATAACTCTCG CCGAGTGTCA GTACAAGGGT CGCCCCGCTC TCAGTGCCCA
GCTCCCCCGG GTATCAGCTG AAACATCAGC TGCCCCTGGG TACGCTCCCG GAGTATCAGC
AAAAGGTTCG CCCCGCCCAC AGTGCGGCTC CCCCGGGTAT CAAAAGAAGG ATCGGCTCCG
CCGCCGGGTC CCCGGGGGAG TTGATAGAAG GGTCCTTCCC ACCCTTTGCC GTCCCCACTC
CTGTGCCTAC GACCCAGGAG CGTGTCAGCC AAAGCATG

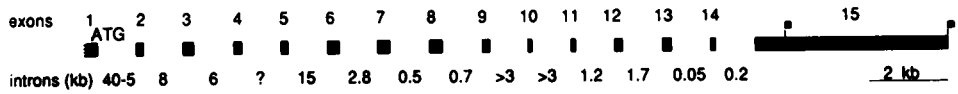
Figure 2. 5′-noncoding sequence of MAO-A. The underlines indicate sequences corresponding to published MAO-A cDNA sequence hMAO A-7 (5), starting from nucleotide no. 1 of the cDNA. The potential TATA box and start site of translation are boxed in.

200 bp are added to the RNA species *in vivo*. These results are consistent with the notion that the two messages for MAO-A arise from alternative use of two polyadenylation sites, which are present in the same exon. The role of this alternative termination of messages in regulation of cellular MAO activities or expression remains to be investigated.

DISCUSSION

This study has demonstrated that the human *MAOA* gene comprises 15 exons distributed over 70 kb of the X chromosome. The exon structure of the human *MAOB* gene, which is located very close to the *MAOA* gene on the same 270 kb Sfi fragment (Z.-Y.Chen, unpublished data) is identical to it (43). Comparative gene mapping studies show that, although X linked in mammals, MAOA is autosomally located in marsupials and monotremes (44). It is therefore of interest that activity levels in human females are consistently higher (about 20%) than those observed in males (45). There is the possibility that the MAO gene(s) are not completely inactivated on the inactive X in female cells, as other loci in this region of Xp have been found to resist this inactivation (46).

The *MAOB* gene is also divided into 15 exons with identical exon boundaries, at least for exons 2−14 (43). Both nucleotide and amino acid sequences of the exons corresponding to the coding regions of the cDNAs for the two genes show extensive homology (5,6). Similarities in amino acids for exon [1] through [15] are: 60% [1], 72% [2], 74% [3], 77% [4], 70% [5], 75% [6], 74% [7], 68% [8], 66% [9], 67% [10], 80% [11], 94% [12], 89% [13], 48% [14], and 60% [15]. Exons with the highest similarities, numbers 11, 12, and 13, are centered around the FAD-covalent binding site which resides in exon 12 (Cys 406



exon	3' end of exon		5' splice site	intron (kb)	3' splice site		5' end of exon	exon
1	...ATT TCA G ...Ile Ser Gly	123 25	gtcagtgtgg...	(40-5)	...tttcctttag	124	GA CTA TCT GCT... Leu Ser Ala...	2
2	...ACT ATA AGG ...Thr Ile Arg	218 56	gtaagtgatt...	(~8)	...ttttttgcag	219	AAT GAG CAT... Asn Glu His...	3
3	...TAT GTC AAG ...Tyr Val Lys	356 102	gtaaggctga...	(6)		357	GGG AAA ACA... Gly Lys Thr...	4
4	...GGG AAG GAG ...Gly Lys Glu	461 137		(?)	...ttctctacag	462	ATT CCA ACT... Ile Pro Thr...	5
5	...TGG ACA AA ...Trp Thr Lys	553 168	gtaagtagac...	(15)	...ctatgaacag	554	G ACT GCT AGG... Thr Ala Arg...	6
6	...GGT GGC CAG ...Gly Gly Gln	695 215	gtatggt?t?...	(2.8)	...cctcctgtag	696	GAA CGG AAG... Glu Arg Lys...	7
7	...CAT TAT GAG ...His Tyr Glu	845 265	gtaactca??...	(.47)	...tgtgttctag	846	TGC AAA TAC... Cys Lys Tyr...	8
8	...AAG AAG G ...Lys Lys Asp	1005 319	gtaggctgct...	(.7)	...cctggttaag	1006	AT TAC TGT GGC... Tyr Cys Gly...	9
9	...ATC ATG GG ...Ile Met Gly	1102 351	gtaggttaga...	(>3)+	...ctctctccag	1103	C TTC ATT CTT... Phe Ile Leu...	10
10	...GAA ATA AG ...Glu Ile Arg	1156 369	gtaagaattt...	(>3)+	...tgttttatag	1157	G AAG AAG AAA... Lys Lys Lys...	11
11	...GCT TTA CAT ...Ala Leu His	1214 388	gtaagaaact...	(1.2)	...tgaactgcag	1215	CCA GTG CAT... Pro Val His...	12
12	...TAT GGA AG ...Tyr Gly Arg	1312 421	gtattacgca...	(1.7)	...tcccttgaag	1313	G GTG ATT CGT... Val Ile Arg...	13
13	...GCT AGG GAG ...Ala Arg Glu	1424 458	gtaagcagga...	(.05)	...tttctttcag	1425	GTC TTA AAT... Val Leu Asn...	14
14	...GAA TCA AAG ...Glu Ser Lys	1487 479	gtaagtttgg...	(.19)	...ccttatctag	1488	GAC GTT CCA... Asp Val Pro...	15
15	...TTGTTAATAA...	3917						

Figure 3. Exon structure and exon-intron junctions of *MAOA* gene. Solid flags above exon No. 15 indicate sites for polyadenylation signals. Numbers to the right or left of nucleotide and amino acid sequences correspond to position numbers reported previously for cDNA clone HM11 (7). ? = not completely sure; > = could not PCR across the intron using primers in flanking exons.

and Cys 397, for *MAOA* and *MAOB*, respectively). This region is immediately followed by two exons with the lowest similarity, number 14 and number 15 at the carboxyl terminus. Exons 11, 12, and 13 may have originated from sequences encoding ancestor flavin-binding polypeptide, while exon 14 and 15 may have evolved to confer different affinities for monoamine substrates between these two isoforms of MAO. It should also be pointed out that, although exon 14, and to a lesser extent, exon 10, are highly divergent between the two forms of MAO, they are highly conserved for each form among different species (7). Evidence exists for the presence, in amphibia, of two forms of monoamine oxidase corresponding in substrate specificity and inhibition properties to the A and B forms observed in mammals (47). It appears that the two closely linked genes in humans represent the products of duplication event occurring >500 MY ago (48). Further studies on the conservation of sequences between A and B forms and between similar forms in different species may enable the elucidation of those functional domains which are important in determining the differences in their substrate specificity and kinetic behavior. Studies of other duplicated genes which have remained very tightly linked over long evolutionary periods have generally established that, where the loci share similar functions, the genes are expressed in different tissues and/or at different developmental times.

There is considerable interest in establishing the extent of genetic variation in MAO genes for two reasons. First, humans inherit widely differing levels of MAO-A (49, 50) and MAO-B (45) activities, and these variations are thought to contribute to predispositions to certain neurological and psychiatric diseases. Second, male adults have been described who carry deletions of both *MAOA* and *MAOB* loci and who exhibit severe neurological problems, as well as manifesting Norrie disease (an X-linked syndrome characterized by congenital blindness) (15–18). These combined symptoms, which are more severe than those reported for typical Norrie disease, are thought to represent a contiguous gene syndrome and suggest that, although complete loss of MAO-A and MAO-B activity is compatible with life, can cause other neurologic problems.

Knowledge of the organization of the *MAOA* gene will enable the construction of oligonucleotide primers that can be employed in the PCR amplification of coding sequences from genomic DNA for analysis of gene mutations and for sequencing from individuals with variations and deficiencies in enzyme activity. Such reagents will also simplify the detection of RFLPs, several of which have been reported for the *MAOA* gene. These include those detected by EcoRV (12, 17), MspI (51) and Fnu4HI (27). In addition, an informative (CA)n repeat polymorphism has been described within the *MAOA* gene (52). Using RFLP analysis of DNA from 40 males whose MAO-A activity measured in cultured skin fibroblasts varied over 200-fold, a strong correlation was observed between allelic status and activity level (27), indicating that the structural locus is a strong determinant of activity levels. There is clearly much to be learned at a variety of levels concerning the relationship between the two forms of the enzyme

and in the differences and similarities in their functional domains. The elucidation of their detailed genomic organization also provides a basis for exploring variations in gene structure associated with disease states.

Note. All intronic sequences obtained during this analysis, as well as the 3′ sequences for the longer form of the MAO-A mRNA encoded in exon 15, have been deposited in EMBL (accession nos X60517–X60541). Following review of this paper, another paper was published describing the exon structure of the human *MAOA* gene (Grimsby et al, 1991). These two papers agree exactly on exon structure, with minor differences in intron sequences, which may reflect polymorphisms.

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