

Characterization of the rat *mas* oncogene and its high-level expression in the hippocampus and cerebral cortex of rat brain

(cellular transformation/receptor/neurotransmitter/guanine nucleotide-binding regulatory protein)

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ABSTRACT The human *mas* oncogene was originally detected by its ability to transform NIH 3T3 cells. We previously showed that the protein encoded by this gene is unique among cellular oncogene products in that it has seven hydrophobic potential transmembrane domains and shares strong sequence similarity with a family of hormone-receptor proteins. We have now cloned the rat homolog of the *mas* oncogene, determined its DNA sequence, and examined its expression in various rat tissues. A comparison of the predicted sequences of the rat and human *mas* proteins shows that they are highly conserved, except in their hydrophilic amino-terminal domains. Our examination of the expression of *mas*, determined by RNA-protection studies, indicates that high levels of *mas* RNA transcripts are present in the hippocampus and cerebral cortex of the brain, but not in other neural regions or in other tissues. This pattern of expression and the similarity of *mas* protein to known receptor proteins suggest that *mas* encodes a receptor that is involved in the normal neurophysiology and/or development of specific neural tissues.

The human *mas* oncogene was originally detected by its ability to render NIH 3T3 cells tumorigenic in nude mice (1). This gene encodes a protein that is unique among cellular oncogene products both in its structure and in its transforming properties (1, 2). The *mas* protein shares a close structural similarity with a group of transmitter and hormone receptors that includes the visual opsins (3), the α_2 -, β_1 -, and β_2 -adrenergic receptors (4–7), the M1 and M2 muscarinic acetylcholine receptors (8, 9), and the substance K receptor (10). The *mas* protein and these receptors exhibit similar hydrophobicity patterns that predict seven distinct transmembrane domains. Furthermore, these sequences all share limited amino acid sequence homology, which suggests that they may have been derived from a common ancestral gene (3, 8–12). In addition to their structural similarity, several of these receptors have been shown to be linked to second-messenger pathways through the activation of guanine nucleotide-binding regulatory proteins (G proteins). Based on these similarities, the *mas* protein may also be a receptor that activates a G protein. To provide additional information on the normal role of the *mas* gene, we have studied its pattern of expression in rat tissues. In this paper, we describe the cloning and DNA sequence[†] of the rat homolog of the *mas* oncogene and demonstrate its high-level expression in the hippocampus and cerebral cortex but not in other regions of the rat brain.

MATERIALS AND METHODS

DNA Sequencing. DNA sequences were determined in both orientations by the dideoxynucleotide chain-terminating method (13).

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RNA Purification. Tissues were dissected from Sprague-Dawley rats (100–150). Brains were dissected according to the procedure of Glowinski and Iversen (14). RNA was purified from rat tissues by published procedures (15, 16). Frozen tissue was solubilized in 7.6 M guanidine hydrochloride (Fluka)/50 mM potassium acetate, pH 5.5, with a Polytron (Brinkmann). Ethanol (0.6 volume) was added and RNA was selectively precipitated at -20°C for several hours. After centrifugation in an International Equipment DPR-6000 centrifuge at $3000 \times g$ for 15 min, the RNA pellet was dissolved in the same guanidine hydrochloride buffer by brief heating at 60°C , extracted with phenol/chloroform (1:1, vol/vol) several times, and precipitated as described above. The RNA pellet was dissolved in the same buffer, precipitated a third time, and dissolved in 10 mM Tris-HCl buffer (pH 7.2).

RNA Probes and Hybridization. SP6 RNA polymerase (Boehringer Mannheim) was used as described (17, 18) to make ^{32}P -labeled RNA transcripts from a clone, pSPRS2, which contains a 406-base-pair fragment of the rat *mas* coding region cloned in the antisense orientation relative to the SP6 promoter. Prior to *in vitro* transcription, the DNA template was linearized with *Pvu* II restriction endonuclease, which cuts at a single site about 600 base pairs downstream from the SP6 promoter. Thus, transcription by SP6 polymerase generated 600-base-long RNA transcripts. This labeled RNA probe was hybridized to 25 μg of total RNA from rat tissue in 30 μl of 80% (vol/vol) formamide/400 mM NaCl/1 mM EDTA/40 mM Pipes, pH 6.4, at 50°C for 12–16 hr. The RNA was then treated with RNases T1 and T2 (Bethesda Research Laboratories) in 300 μl of 50 mM sodium acetate/2 mM EDTA/100 mM sodium chloride, pH 5.0, for 60 min at 30°C to digest unhybridized RNA. Samples were then extracted with phenol/chloroform (1:1) three times, precipitated with 8 μg of tRNA, and electrophoresed in a 4% polyacrylamide gel containing 8 M urea. The gel was dried and exposed to Kodak XAR-5 film.

RESULTS

Cloning and Sequence of the Rat *mas* Gene. We cloned the rat homolog of the *mas* oncogene primarily to use it as a probe for studying *mas* expression in rat tissues. Blot analysis of *Eco*RI-cleaved rat genomic DNA showed that a single 5-kilobase fragment hybridized to a nick-translated, ^{32}P -labeled human *mas* probe (data not shown). To clone this DNA sequence, *Eco*RI-cleaved rat DNA was fractionated by electrophoresis in a 1% agarose gel and the DNA was purified from the region of the gel that contained the hybridizing DNA

Abbreviation: G protein, guanine nucleotide-binding regulatory protein.

[†]The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03823).

fragment. A library was constructed from this DNA in the λ gt10 vector (19), and clones that hybridized to a human *mas* probe were isolated from this library. One of these clones, pRM1, was sequenced and found to contain an open reading frame encoding a protein that is highly homologous to the human *mas* protein. Like the human *mas* gene, the rat homolog appears not to have introns in its coding region. This is a property shared by several other genes encoding members of this family of receptors.

Fig. 1 shows a comparison of the DNA and predicted amino acid sequences of the rat and human *mas* gene coding regions. The rat and human protein sequences are almost identical except in their amino- and carboxyl-terminal domains. The DNA sequences are 85% identical in the coding regions, and strong homology extends 36 base pairs upstream and 80 base pairs downstream from the coding regions. The rat sequence codes for a protein with 324 amino acid residues, whereas the human *mas* protein has 325 amino acid residues. This difference in length is due to the presence of one

additional amino acid in the amino-terminal domain of the human *mas* protein that is absent in the rat *mas* protein. The rat and human proteins share 90% amino acid identity overall, with most of the differences occurring in the amino-terminal hydrophilic domains, which are only 52% identical. The sequence homology that exists between the amino-terminal domains reflects, in part, the conservation of three potential N-glycosylation sites.

The structural similarity between the rat and human *mas* proteins suggests that their interactions with ligands and effector molecules have also been conserved. The hydrophobic domains of receptors that are related to the *mas* protein are thought to be involved in ligand binding (20–22). In rhodopsin it has been shown that the photoactive molecule, retinal, binds within the hydrophobic core of the protein (22). If the ligand for the *mas* protein also binds within the hydrophobic core, then the high conservation of the hydrophobic domains of the human and rat *mas* proteins argues that they interact with similar or identical ligands.

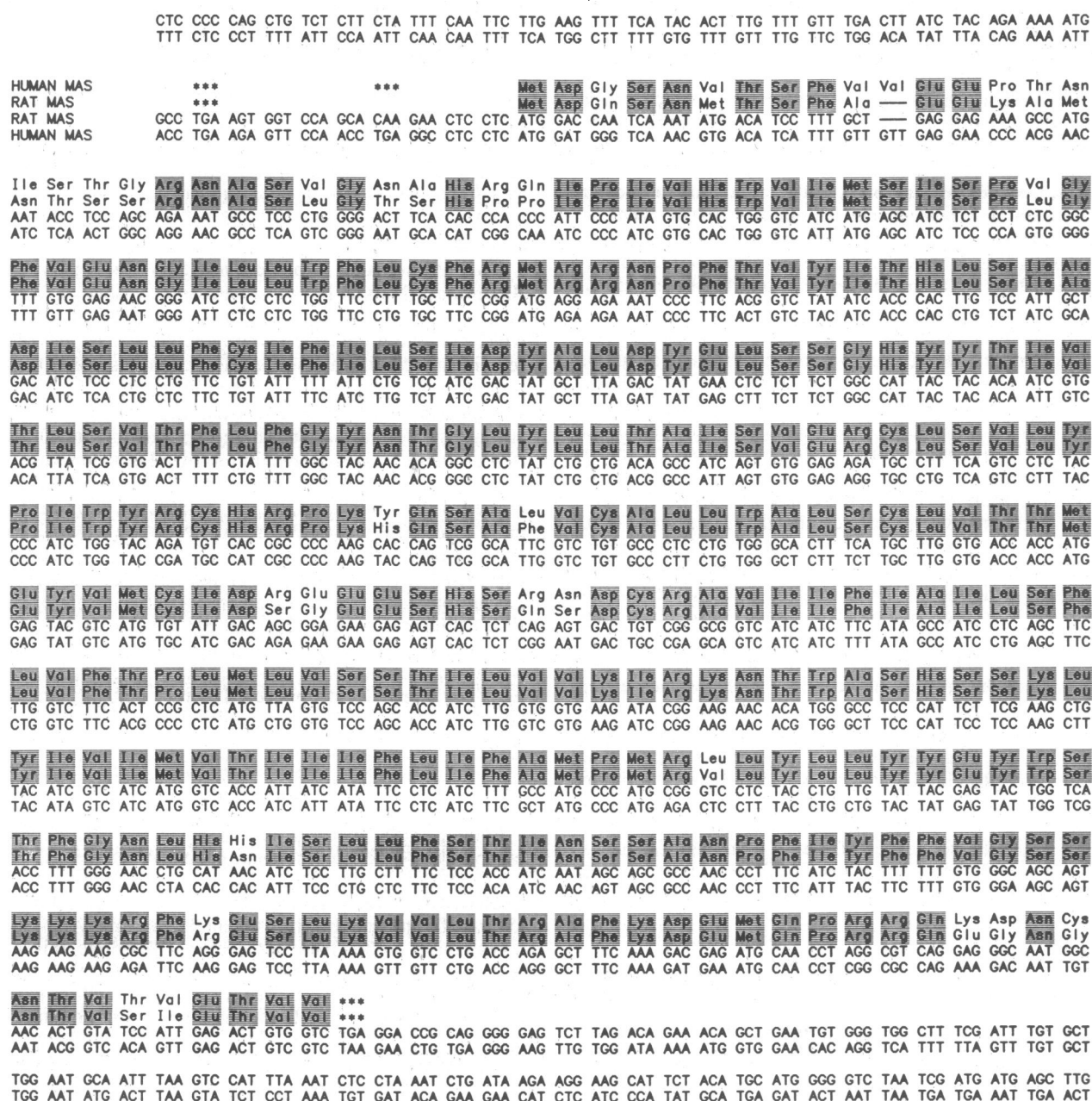


FIG. 1. Comparison of the DNA and predicted amino acid sequences of the rat and human *mas* gene coding regions. Amino acid residues that are identical in corresponding positions of the two proteins are shaded.

The rat and human *mas* proteins are functionally similar in their ability to transform NIH 3T3 cells. Clones containing the Moloney murine leukemia virus promoter linked to the coding region of either the rat or the human gene are capable of inducing foci at similar frequencies (data not shown). Cells transformed by these constructs have a normal morphology but are very tumorigenic in nude mice (2). Although these data suggest that the particular sequence differences in the amino- and carboxyl-terminal domains do not grossly alter the function of *mas* protein, they do not rule out the possible importance of these domains in the normal physiology of *mas* protein.

Detection of *mas* RNA Transcripts. To assay *mas* RNA levels in rat tissues, we used a sensitive RNA-RNA hybridization procedure that can detect less than 1 copy of mRNA per cell (17, 18). High-specific-activity antisense transcripts were made from a short region of the rat *mas* coding sequence by using SP6 RNA polymerase. This ³²P-labeled RNA probe was then hybridized to total RNA from rat tissue in solution. The unhybridized single-stranded RNA was digested with RNases and the protected double-stranded RNA was resolved in a polyacrylamide gel. Fig. 2 shows the results of a screen for *mas* RNA transcripts in different rat tissues by this method. This figure shows that *mas* is expressed in rat brain but not at detectable levels in other tissues including pancreas, small intestine, heart, spleen, kidney, skeletal muscle, skin, and liver.

To examine the distribution of *mas* expression in the brain, we performed a similar screen with RNA from different regions of the rat brain. Fig. 3 shows that *mas* RNA transcripts are present at a high level in the hippocampus and at

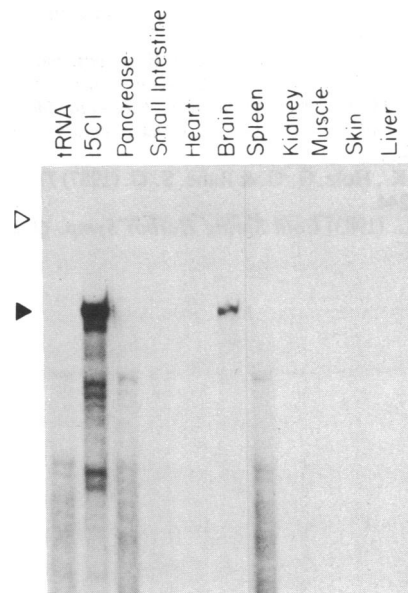


FIG. 2. Expression of *mas* RNA in rat tissues. RNA was purified and analyzed for *mas* RNA transcripts by RNase protection. Open triangle indicates the position of migration in the gel of the full-length 600-base RNA probe. The strong signals, indicated by the solid triangle, represent the 406-base protected region of the RNA probe that hybridized to rat *mas* RNA transcripts. The first lane shows the result of using tRNA in the hybridization as a negative control. The second lane shows results for RNA from 15C1, a transfected NIH 3T3 (murine) cell line that expresses the rat *mas* gene. The other lanes show results for RNA purified from various rat tissues. Of these tissues, only brain shows a signal. Although faint signals are sometimes seen with RNA from the other tissues, we have shown in separate experiments that they are totally eliminated by treatment of the RNA samples, prior to hybridization, with RNase-free DNase I (Worthington) followed by precipitation in 4 M LiCl to remove residual DNA.

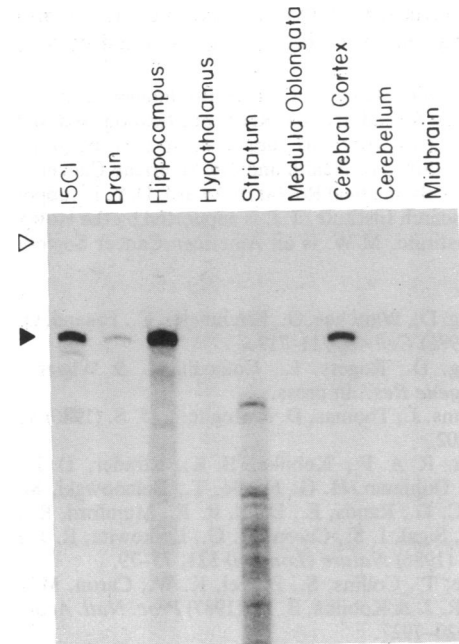


FIG. 3. Expression of *mas* RNA in rat brain regions. RNA was purified and analyzed for *mas* RNA transcripts by RNase protection. Triangles indicate the positions of migration of the full-length RNA probe (open triangle) and the protected probe fragment (solid triangle), as in Fig. 2. The first lane represents results for RNA from 15C1 (see Fig. 2 legend). The other lanes show results for RNA from total rat brain and various brain regions.

approximately one-third that level in the cerebral cortex, but not at appreciable levels in hypothalamus, striatum, medulla oblongata, cerebellum, or midbrain. By comparing levels of *mas* RNA transcripts in the hippocampus with levels in a transfected cell line, we estimated that *mas* transcripts represent roughly 0.005% of mRNA in the hippocampus.

DISCUSSION

The expression of *mas* in the hippocampus and cerebral cortex of the brain suggests that the *mas*-encoded protein may be a neurotransmitter receptor that is specifically localized in these neural regions. The idea that some oncogenes may encode neurotransmitter receptors is supported by observations that certain monoamines and neuropeptides, such as serotonin, substance P, and substance K, are mitogenic in culture (23, 24). The binding sites of most neurotransmitters have broad distributions that differ from that of *mas* RNA transcripts. Nevertheless, ligands that bind to certain pharmacologically distinct receptors, such as the phencyclidine receptor (25, 26) and the *N*-methyl-D-aspartate and quisqualate receptors (27–29), have high densities of binding sites in regions of the hippocampus and cerebral cortex. It is conceivable that the *mas* gene encodes one of these known receptors.

A number of neurotransmitter receptors related to *mas* protein are thought to be coupled to G proteins. G proteins function as intermediaries in transmembrane signaling pathways to modulate a variety of intracellular responses. Examples of G-protein modulation of biochemical pathways include the stimulation and inhibition of adenylate cyclase (30, 31), stimulation of phosphatidylinositol metabolism (32, 33), regulation of K⁺ and Ca²⁺ channel activities (34), and stimulation of retinal cGMP phosphodiesterase activity (35). G proteins may be involved in transducing signals from a number of hormones and neurotransmitters to effect other cellular responses. From the similarity of *mas* protein to a

number of receptors that are coupled to G proteins, we suspect that *mas* function is also mediated by a G protein.

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