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Characterization of Two New Human Oncogenes

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The first oncogenes discovered were the transforming genes of the oncogenic viruses (reviewed by Bishop 1985). The subsequent discovery that the oncogenes of retroviruses were derived from normal host cellular genes provided the first direct evidence that cellular genomes contain genes with transforming potential. More recently, the development of techniques for DNA transfer in eukaryotic cells led to the discovery of cellular transforming genes in tumor cells by their ability to induce foci of transformed NIH-3T3 cells (reviewed by Land et al. 1983). Several new oncogenes have been discovered this way, including N-*ras* (Shimizu et al. 1983), *met* (Cooper et al. 1984), *neu* (Bargmann et al. 1986), and possible others (Goubin et al. 1983; Lane et al. 1984; Takahashi et al. 1985).

We and others have developed a new assay for human oncogenes based on the tumorigenicity of cotransfected NIH-3T3 cells in nude mice, using human tumor DNA as donor (Blair et al. 1982; Fasano et al. 1984). While we sometimes can detect genetic abnormalities in donor DNA with this method, we have also observed that de novo activation of proto-oncogenes due to rearrangement or gene amplification can occur following gene transfer. Here, we describe the isolation and characterization of two human oncogenes, *mcf3* and *mas1*, detected by the cotransfection and tumorigenicity assay.

The first gene, *mcf3*, encodes a tyrosine kinase with a potential transmembrane domain. It arose from the human *ros1* gene by a rearrangement introduced during transfection, which deleted the putative extracellular domain of *ros1* (Birchmeier et al. 1986). The human *ros1* is the closest homolog of the v-*ros* oncogene, which is the transforming gene of the avian UR2 retrovirus (Neckameyer and Wang 1985). Structurally, *mcf3* is thus similar to a class of oncogenes whose cellular analogs presumably encode growth factor receptors. As a first step toward the characterization of the normal *ros1* gene, we have analyzed its expression in a variety of human tumor cell lines. *ros1* is expressed in glioblastoma and astrocytoma cells, and the amount of *ros1* expression seems to correlate with the extent of transformation of this cell type.

The second gene, *mas1*, encodes a protein with seven hydrophobic regions that are potential transmembrane domains, suggesting that *mas* is an integral membrane protein (Young et al. 1986). The structure of the *mas* protein is unique among cellular oncoproteins and may represent a new functional class.

RESULTS AND DISCUSSION

Cotransfection and Tumorigenicity Assay

In brief, the new assay is performed as follows. NIH-3T3 cells are cotransfected with human cellular DNA and DNA of the pKOneo plasmid, which renders animal cells resistant to the antibiotic G418 (Van Daren et al. 1984). In each experimental group, about 10^3 G418-resistant colonies containing about 10^4 cells each are present after 2–3 weeks. These are pooled and injected into one nude mouse. The time of the first appearance of tumors and their subsequent growth is noted. We call tumors resulting from cells exposed to total human DNA "primary" tumors. DNAs prepared from the primary tumors are used again in the cotransformation and tumorigenicity assay to obtain secondary tumors. DNAs from primary and secondary tumors are then compared by Southern blotting for the presence of a common set of human repetitive sequences. Genomic libraries of the secondary tumors are constructed, and screened for the presence of repetitive human sequences (Benton and Davis 1977). Both the *mcf3* and *mas* genes, which were isolated by the strategy described above, were found to be rearranged and amplified in the nude mouse tumors. In both cases the rearrangements were shown to be introduced during or after gene transfer (Birchmeier et al. 1986; Young et al. 1986).

Rearrangements and amplifications also occur in the standard NIH-3T3 focus assay and have been reported to lead to proto-oncogene activation (Takahashi et al. 1985). In our experience, such events are rare in the focus assay but common with the cotransfection and tumorigenicity assay. The cotransfection and tumorigenicity assay, therefore, may be unreliable for the detection of oncogenes in tumor DNAs. However, it may be a good method for searching for proto-oncogenes which can be activated by rearrangement or amplification.

Structural Analysis of the *mcf3* Oncogene

The *mcf3* oncogene was isolated by molecular cloning, and its structure was compared with the structure of its normal counterpart in human placental DNA. We found that the *mcf3* oncogene was a product of a major DNA rearrangement which involved the fusion of at least three separate fragments of DNA (Birch-

meier et al. 1986). DNA cotransfection studies indicated that this rearrangement spanned functionally important domains of *mcf3*. The peculiar rearrangement associated with the *mcf3* oncogene was shown to be present only in DNA isolated from transfected and tumorigenic cells, but not in the original donor DNA. Thus, a functional *mcf3* gene was created by a rearrangement introduced during or after gene transfer. It is clearly a very large gene, which extends over nearly 70 kbp of DNA.

To analyze the *mcf3* oncogene further, cDNAs to the transcript of the gene were isolated and the sequence of the open reading frame in the cDNA was determined. Computer analysis of the amino acid sequence indicated that the carboxy-terminal part of the *mcf3* protein sequence was similar to that of all oncogenes encoding tyrosine kinases and closely related to v-*ros* (Neckameyer and Wang 1985). For comparison, the amino acid sequences of *mcf3*, v-*ros*, and other protein kinases are shown in Figure 1. From amino acids 51 to 370 of *mcf3*, 75% homology exists between the *mcf3* and v-*ros* proteins. Only at the carboxyl terminus do they differ considerably, where the *mcf3*-encoded protein contains 99 additional amino acids not found in the v-*ros* protein. As has been noted before, the tyrosine kinase most closely related to v-*ros* is the insulin receptor (Ullrich et al. 1985). This is also true for *mcf3*, which shows 40%, 33%, and 31% homology to the insulin receptor, v-*src*, and the EGF receptor, respectively, between amino acid positions 51–370. In partic-

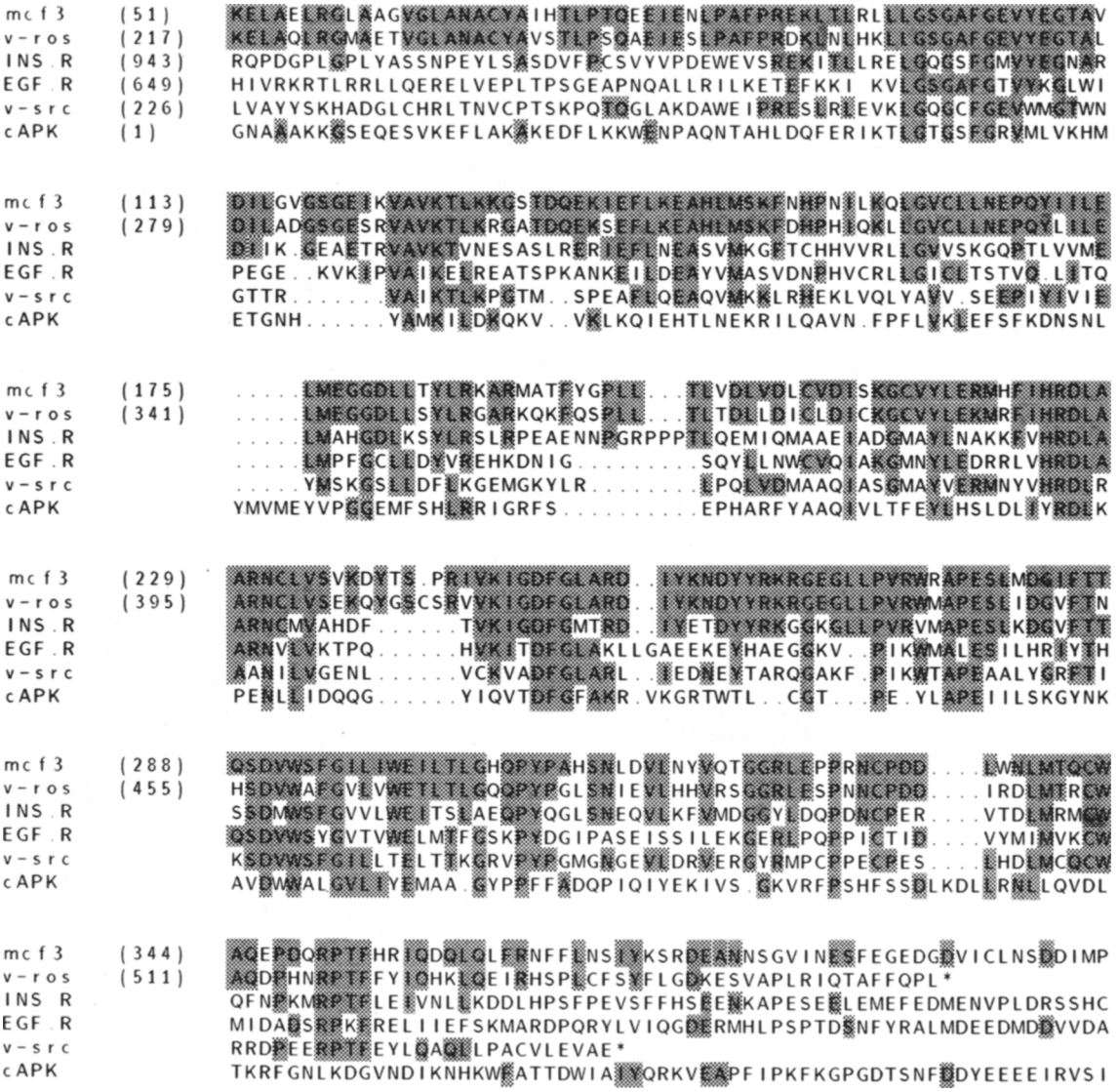

Figure 1. Comparison of the putative tyrosine kinase domain of the *mcf3* gene product with the corresponding domains of various other protein kinases. The predicted amino acid sequences of the putative catalytic domain of the gene product of *mcf3*, v-*ros* (Neckameyer and Wang 1985), the human insulin receptor (INS.R) (Ullrich et al. 1985), EGF receptor (EGF.R) (Ullrich et al. 1984) and v-*src* (Tatsuo et al. 1982) genes, and the bovine cAMP-dependent protein kinase (cAPK) (Shoji et al. 1981) are shown. All residues shared between *mcf3* and one or more of the other kinases are shaded. The numbers to the left refer to the first amino acid shown in the corresponding line. The asterisks indicate the termination codons of the indicated proteins.

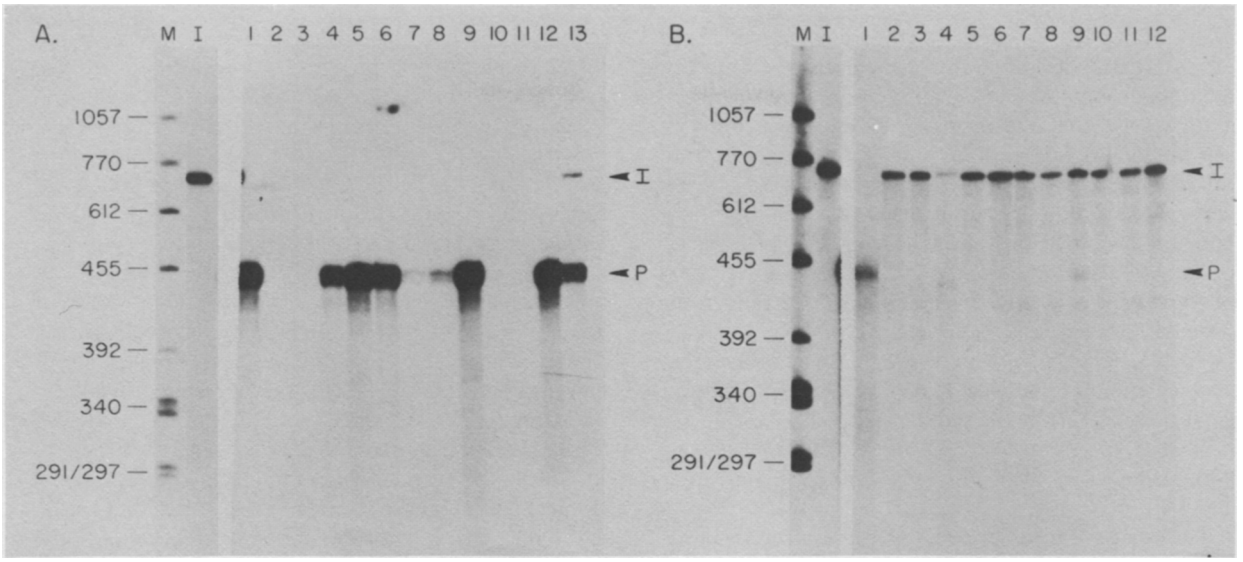

Figure 2. Expression of *ros1* in human tumor cell lines. Total RNA (20 μg) prepared by the guanidinium/CsCl method (Ullrich et al. 1977) was hybridized overnight at 52°C to approximately 1 fmole of a ^{32}P-labeled RNA transcript produced by Sp6 polymerase (sp. act. 8×10^7 cpm/pmole) (Melton et al. 1984). The hybridization mixture was diluted 10-fold with buffer containing 50 mM Na acetate (pH 4.5), 2 mM EDTA, and 100 mM NaCl and treated with 10 U of RNase T2 for 1 hr at 30°C. The nucleic acids were precipitated with EtOH, and analyzed on a 5% acrylamide gel containing 8 M urea which was autoradiographed. The labeled RNA transcript contained sequences of 480 and 270 bases in length homologous to *mcf3* and plasmid sequences, respectively. *ros1* and *mcf3* mRNA therefore protected a fragment of 480 bases in length from RNase T2 digestion. (Lanes *M*) DNA size marker; the lengths of the fragments are indicated. (Lanes *1*) ^{32}P-Labeled RNA probe. The arrows indicate the labeled RNA transcript without RNase treatment (I) and the fragment protected from RNase T_2 digestion by *mcf3* or *ros1* mRNA (P). (*A*) (Lane *1*) 1 μg of RNA from *mcf3*-transformed NIH-3T3 and 19 μg of carrier RNA. (Lane *2*) 20 μg RNA from SK-N-SH (neuroblastoma). (Lane *3*) 20 μg RNA from SK-N-ML (neuroblastoma). (Lane *4*) 20 μg of RNA from U105 MG (glioblastoma–astrocytoma grade III). (Lane *5*) 20 μg of RNA from U118 MG (glioblastoma multiforme). (Lane *6*) 20 μg of RNA from U138 MG (glioblastoma). (Lane *7*) 20 μg of RNA from U178 MG (glioblastoma–astrocytoma grade II). (Lane *8*) 20 μg of RNA from U251 MG (glioblastoma–astrocytoma grade I). (Lane *9*) 20 μg of RNA from U343 MG (glioblastoma multiforme). (Lane *10*) 20 μg of RNA from A172 (glioblastoma). (Lane *11*) 20 μg of RNA from A382 (glioblastoma). (Lane *12*) 20 μg of RNA from SW 1088 (glioblastoma). (Lane *13*) 20 μg of RNA from SW 1783 (glioblastoma–astrocytoma grade III). (*B*) (Lane *1*) 0.05 μg of RNA from *mcf3*-transformed NIH-3T3 and 20 μg of carrier RNA. (Lane *2*) 20 μg of RNA from 734B (adenocarcinoma, breast). (Lane *3*) 20 μg of RNA from MCF7 (adenocarcinoma, breast). (Lane *4*) 20 μg of RNA from VM-Cub2 (carcinoma, bladder). (Lane *5*) 20 μg of RNA from TCCsup (carcinoma, bladder). (Lane *6*) 20 μg of RNA from 575A (carcinoma, bladder). (Lane *7*) 20 μg of RNA from 486P (carcinoma, bladder). (Lane *8*) 20 μg of RNA from RD2 (rhabdomyosarcoma). (Lane *9*) 20 μg of RNA from Caki 1 (carcinoma, kidney). (Lane *10*) 20 μg of RNA from SK-N-EP (Wilm's tumor). (Lane *11*) 20 μg of RNA from Wilv 1 (Wilm's tumor). (Lane *12*) 20 μg of RNA from human placenta.

ular, a stretch of 75% homology to the insulin receptor exists between positions 255 and 288 in *mcf3*, as compared with 28% and 37% to the EGF receptor and v-*src*, respectively. This stretch of high homology can be aligned with positions 188–214 in the cAMP-dependent protein kinase. Since Cys-198 of the cAMP-dependent protein kinase is protected from chemical modification by peptide substrates, this region has been implicated in substrate binding (Branson et al. 1982). The high degree of homology between the amino acid sequences of *mcf3* and the insulin receptor in this putative substrate-binding domain might indicate a similar substrate specificity for the tyrosine kinase activities of these two proteins.

The close homology between the chicken-derived v-*ros* gene and the common part of the *mcf3* cDNAs suggests that the 3′ portion of the *mcf3* locus derives from the human counterpart of the v-*ros* gene. To determine whether this was indeed the case, we performed Southern analyses of total human DNA under conditions of low stringency with two probes, a v-*ros* and a *mcf3* cDNA fragment, which encode roughly analogous sequences of the *ros* proteins. Both probes hybridized most strongly to the same fragment in total human DNA also present in the *mcf3* locus. We conclude, therefore, that a major portion of the *mcf3* coding sequence is derived from the gene in humans most closely related to the v-*ros* gene. We call this gene *ros1*, since other human genes related to the v-*ros* or the *mcf3* gene may exist.

Five of the oncogenes known to encode oncogenic tyrosine kinases, v-*ros* (Neckameyer and Wang 1985), v-*erbB* (Yamamoto et al. 1983), v-*fms* (Hampe et al. 1984), *neu* (Bargmann et al. 1986), and *trk* (Martin-Zanca et al. 1986), have hydrophobic potential membrane-spanning domains. The membrane-spanning domains of these proteins are always encoded 5′ to sequences encoding the kinase domain. Inspection of the *mcf3* nucleotide sequence shows that it can encode a highly hydrophobic stretch of 21 amino acids immedi-

ately followed by a stretch rich in positively charged amino acids. These features are commonly found in membrane-spanning domains. We wanted to know exactly which portion of the *mcf3* cDNA was encoded by the human *ros1* gene and which parts derived from other sequences fused by rearrangement to *ros1*. By combined restriction endonuclease analysis, Southern blotting, and hybridization with synthetic oligonucleotides, the positions of exons in the *ros1*-derived part of the *mcf3* locus close to the point of rearrangement were determined, and their sequence was partially established. Thus, we found that the rearrangement which created the oncogenic *mcf3* gene deleted all but eight amino acids of the putative extracellular domain of *ros1*. DNA pieces of unknown origin replaced this part of the *ros1* gene in the *mcf3* locus (Birchmeier et al. 1986).

The deletion of the extracellular domain may be an important event in the activation of the oncogenic potential of the *ros1* gene. Similar events have been observed previously for the v-*erbB* (Downward et al. 1984) and the c-*erbB* (Nilsen et al. 1985) genes in avian leukosis virus-induced erythroblastosis. Insertion of the provirus into the middle to the c-*erbB* gene leads to the production of a truncated *erbB* transcript which encodes only 64 amino acids of the extracellular domain but an intact membrane-spanning and intracellular domain. Similarly, *trk* seems to have been formed by a somatic rearrangement that replaced the extracellular domain of a putative transmembrane receptor with the first 221 amino acids of a nonmuscle tropomyosin protein (Martin-Zanca et al. 1986). We have not analyzed the nature of the sequences in *mcf3* that have replaced the extracellular domain of the *ros1* gene. However, we should be cautious in concluding that the loss of the extracellular sequences has led to the activation of *ros1*. Although we could not detect any gross structural differences between the DNA of *mcf3* and *ros1* coding for

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      GGATCCAGAAGGGTCATTCAATCAGTTCTCAGTCTTATCAGGTCTAAGTTCCTTTCTTATCAGGTCCTAAAGG
CCTAATCTTATCATTGTGACAAAGATAACTGTAGAGTCTGTTAAACTTTTTTTTAATAACATGAAGATTATGATTTATA
GCTGAATTTCTCCCTTTTATTCCAATTCAACAATTTTCATGGCTTTTTGTGTTTGTTTTGTTCTGGACATATTTACAGA

                          ***                 MET ASP GLY SER ASN VAL THR SER PHE VAL      10
AAATTACCTGAAGAGTTCCAACC TGA GGC CTC CTC ATG GAT GGG TCA AAC GTG ACA TCA TTT GTT

VAL GLU GLU PRO THR ASN ILE SER THR GLY ARG ASN ALA SER VAL GLY ASN ALA HIS ARG      30
GTT GAG GAA CCC ACG AAC ATC TCA ACT GGC AGG AAC GCC TCA GTC GGG AAT GCA CAT CGG

GLN ILE PRO ILE VAL HIS TRP VAL ILE MET SER ILE SER PRO VAL GLY PHE VAL GLU ASN      50
CAA ATC CCC ATC GTG CAC TGG GTC ATT ATG AGC ATC TCC CCA GTG GGG TTT GTT GAG AAT

GLY ILE LEU LEU TRP PHE LEU CYS PHE ARG MET ARG ARG ASN PRO PHE THR VAL TYR ILE      70
GGG ATT CTC CTC TGG TTC CTG TGC TTC CGG ATG AGA AGA AAT CCC TTC ACT GTC TAC ATC

THR HIS LEU SER ILE ALA ASP ILE SER LEU LEU PHE CYS ILE PHE ILE LEU SER ILE ASP      90
ACC CAC CTG TCT ATC GCA GAC ATC TCA CTG CTC TTC TGT ATT TTC ATC TTG TCT ATC GAC

TYR ALA LEU ASP TYR GLU LEU SER SER GLY HIS TYR TYR THR ILE VAL THR LEU SER VAL     110
TAT GCT TTA GAT TAT GAG CTT TCT TCT GGC CAT TAC TAC ACA ATT GTC ACA TTA TCA GTG

THR PHE LEU PHE GLY TYR ASN THR GLY LEU TYR LEU LEU THR ALA ILE SER VAL GLU ARG     130
ACT TTT CTG TTT GGC TAC AAC ACG GGC CTC TAT CTG CTG ACG GCC ATT AGT GTG GAG AGG

CYS LEU SER VAL LEU TYR PRO ILE TRP TYR ARG CYS HIS ARG PRO LYS TYR GLN SER ALA     150
TGC CTG TCA GTC CTT TAC CCC ATC TGG TAC CGA TGC CAT CGC CCC AAG TAC CAG TCG GCA

LEU VAL CYS ALA LEU LEU TRP ALA LEU SER CYS LEU VAL THR THR MET GLU TYR VAL MET     170
TTG GTC TGT GCC CTT CTG TGG GCT CTT TCT TGC TTG GTG ACC ACC ATG GAG TAT GTC ATG

CYS ILE ASP ARG GLU GLU GLU SER HIS SER ARG ASN ASP CYS ARG ALA VAL ILE ILE PHE     190
TGC ATC GAC AGA GAA GAA GAG AGT CAC TCT CGG AAT GAC TGC CGA GCA GTC ATC ATC TTT

ILE ALA ILE LEU SER PHE LEU VAL PHE THR PRO LEU MET LEU VAL SER SER THR ILE LEU     210
ATA GCC ATC CTG AGC TTC CTG GTC TTC ACG CCC CTC ATG CTG GTG TCC AGC ACC ATC TTG

VAL VAL LYS ILE ARG LYS ASN THR TRP ALA SER HIS SER SER LYS LEU TYR ILE VAL ILE     230
GTC GTG AAG ATC CGG AAG AAC ACG TGG GCT TCC CAT TCC TCC AAG CTT TAC ATA GTC ATC

MET VAL THR ILE ILE ILE PHE LEU ILE PHE ALA MET PRO MET ARG LEU LEU TYR LEU LEU     250
ATG GTC ACC ATC ATT ATA TTC CTC ATC TTC GCT ATG CCC ATG AGA CTC CTT TAC CTG CTG

TYR TYR GLU TYR TRP SER THR PHE GLY ASN LEU HIS HIS ILE SER LEU LEU PHE SER THR     270
TAC TAT GAG TAT TGG TCG ACC TTT GGG AAC CTA CAC CAC ATT TCC CTG CTC TTC TCC ACA

ILE ASN SER SER ALA ASN PRO PHE ILE TYR PHE PHE VAL GLY SER SER LYS LYS LYS ARG     290
ATC AAC AGT AGC GCC AAC CCT TTC ATT TAC TTC TTT GTG GGA AGC AGT AAG AAG AAG AGA

PHE LYS GLU SER LEU LYS VAL VAL LEU THR ARG ALA PHE LYS ASP GLU MET GLN PRO ARG     310
TTC AAG GAG TCC TTA AAA GTT GTT CTG ACC AGG GCT TTC AAA GAT GAA ATG CAA CCT CGG

ARG GLN LYS ASP ASN CYS ASN THR VAL THR VAL GLU THR VAL VAL ***                     325
CGC CAG AAA GAC AAT TGT AAT ACG GTC ACA GTT GAG ACT GTC GTC TAA GAACTGTGAGGGAAG

TTGTGGATAAAAATGGTGGAACACAGGTCATTTTTAGTTTGTGCTTGGAATATGACTTAAGTATCTCCTAAATGTGATA
CAGAAGAACATCTCATCCCATATGCATGAGATACTAATTAATGATGAAA
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Figure 3. Nucleotide sequence and predicted amino acid sequence of the *mas* gene. This sequence was determined from the genomic clone pMS422 and homologous cDNA clones, which contain the long open reading frame. The amino acid sequence deduced from the coding region is shown above the DNA sequence. The in-frame stop codons are indicated by asterisks. The numbers on the right are amino acid coordinates.

the carboxy-terminal part of the protein, we would not have detected any subtle changes such as point mutations or small deletions by our methods of analysis. Moreover, the rearranged *ros1* gene is very highly amplified in all *mcf3*-transformed NIH-3T3 cells we examined. We cannot presently assess the relative contributions of rearrangement and amplification of this gene to its oncogenicity.

Expression of *ros1*

The majority of known oncogenes encode proteins with tyrosine-specific protein kinase activity. However, potential membrane-spanning domains amino-terminal to kinase domains are found only in v-*erbB* (Yamamoto et al. 1983), v-*fms* (Hampe et al. 1984), *neu* (Bargmann et al. 1986), *trk* (Martin- Zanca et al. 1986), and the v-*ros* and cellular *ros* genes (Neckameyer and Wang 1985; Neckameyer et al. 1986). The cellular analogs of the v-*erbB* and the v-*fms* genes probably encode the receptors for the epidermal growth factor and macrophage colony-stimulating factor, respectively (Downward et al. 1984; Sherr et al. 1985). Thus, the cellular *ros* gene very likely encodes a hormone receptor as well.

As a first step toward the molecular characterization of this potential growth factor receptor we have analyzed the expression of *ros1* in human tumor cell lines. We have used liquid hybridization of total cellular RNA to Sp6-produced RNA probes of high specific activities, followed by digestion of the hot hybridized probe by RNase (Melton et al. 1984). This technique proves to be highly specific and very sensitive. In a survey of 40 different cell lines (see Fig. 2a,b; data not shown), we found *ros1* to be expressed in astrocytoma and glioblastoma cells at levels ranging between 10–200 messenger molecules per cell. In contrast, we found *ros1* to be expressed not at all or at very low levels in the remainder of cell lines. From astrocytomas arise a variety of tumors ranging from benign to highly malignant. The malignant forms include the highly malignant glioblastoma multiforme. Interestingly, we find a good correlation between *ros1* expression and the degree of malignancy. This raises the possibility that the expression of *ros1* contributes to the malignant phenotype of this particular cell type, and may be useful in the classification and diagnosis of this type of cancer.

Structure of the *mas* Oncogene

The *mas* oncogene was isolated by cosmid cloning. One cosmid, pMAS1, was shown to contain the entire transforming gene since it scored positive in the cotransfection and tumorigenicity assay (Young et al. 1986). To define the regions of pMAS1 essential for transforming activity, various restriction endonuclease digests of pMAS1 were tested by the NIH-3T3 focus assay. To define the transcription unit and coding potential of the *mas* gene, we cloned cDNAs complementary to *mas* mRNA and sequenced them. We found a open reading frame of 975 bp. The first ATG in this reading frame is preceded by an in-frame stop codon at position −12. The entire open reading frame was contained on a single cDNA clone which was used to construct an expression plasmid containing the coding region of *mas* under the control of the SV40 promoter. NIH-3T3 cells transfected with this construct are tumorigenic in nude mice. Comparison of the restriction endonuclease cleavage sites and sequence analysis of genomic and cDNA clones indicated that the cDNA is entirely colinear with genomic sequences. The nucleotide and predicted amino acid sequence of the encoded protein are shown in Figure 3.

Comparison of Southern blots of normal human DNA with DNA derived from the nude mouse primary tumor revealed a difference in the size and intensity of *Eco*RI restriction fragments homologous to *mas*, suggesting that the *mas* gene was rearranged and amplified in transformants. To determine the nature of the DNA rearrangement, the clone pHM2 containing the normal human homolog of the *mas* gene was isolated

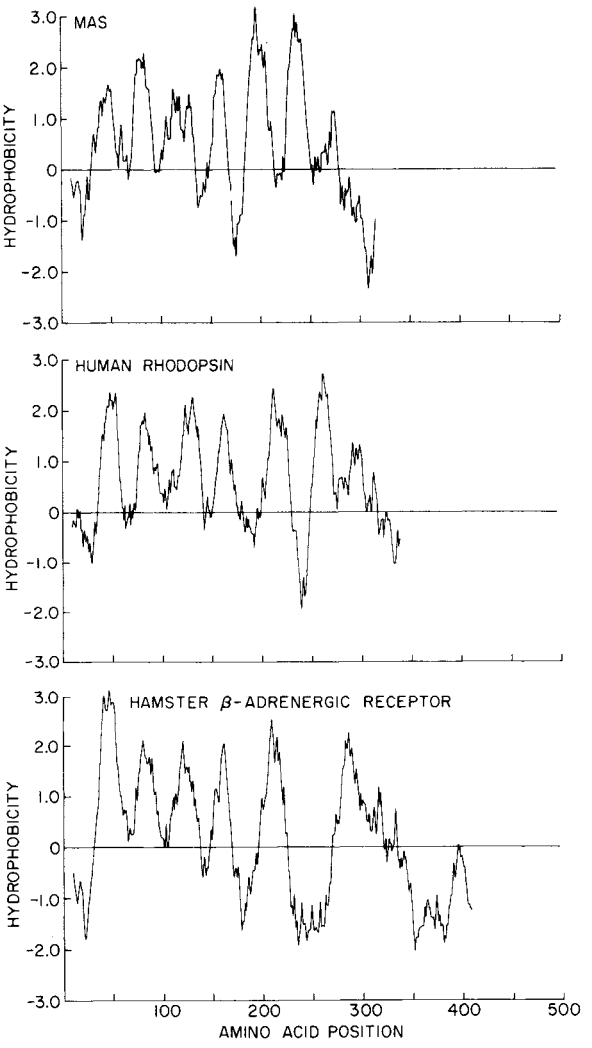

Figure 4. Hydrophobicity plots of human *mas*, human rhodopsin, and the hamster β-adrenergic receptor. Average hydrophobic values were determined for spans of 19 residues using the method and hydrophobic values of Kyte and Doolittle (1982). Regions with positive values are hydrophobic.

from a human placenta cosmid library. Comparison of maps of restriction endonuclease sites in pHM2 and pMAS1 confirmed a break in homology localized in the 5′ noncoding region of pMAS1 essential for transformation of NIH-3T3 cells by the *mas* gene (Young et al. 1986). This suggests that the rearrangement found in the transformant is of functional significance.

We do not understand precisely how the *mas* gene became activated during gene transfer. In transformants, the *mas* gene has been rearranged, but this rearrangement does not appear to involve the coding domain. It is clear that we can activate the oncogenic potential of a normal placental allele by reconstructing a chimeric gene that replaces the normal 5′ sequences with sequences 5′ to the coding region of the rearranged gene. This chimeric gene leaves intact the long open reading frame of the placental allele. Thus, it seems likely that transformation by *mas* results from inappropriate expression of a normal gene product.

Predicted Primary and Secondary Structure of the *mas* Protein

The complete nucleotide sequence of the coding region was determined from *mas* cDNAs. The deduced amino acid sequence does not share strong homology with any published sequence. Analysis of the hydrophobicity plot of the sequence by the method of Kyte and Doolittle (1982) reveals that the *mas* protein has seven distinct hydrophobic regions (see Fig. 4). Each of the hydrophobic regions is separated by hydrophilic regions that contain a predicted β-turn secondary structure (Chou and Fasman 1978). Both the amino- and carboxy-terminal ends of the *mas* protein are hydrophilic. This analysis strongly suggests that the *mas* protein is an integral membrane protein with many transmembrane domains. While the structure of *mas* is unlike the canonical structure of most of the known hormone receptors, it is similar to a class of proteins that includes visual rhodopsin and the β-adrenergic receptor. The similarity of the hydrophobicity plots of these proteins, shown in Figure 4, is certainly striking. Indeed, when the amino acid sequences of *mas*, the visual opsins, and the β-adrenergic receptor are aligned, certain homologies appear (see Fig. 5). Particularly noteworthy is homology in the seventh transmembrane domain.

Visual rhodopsin can be viewed as a retinoid receptor that transduces the energy of photons into chemical energy used to activate transducin, an intracellular

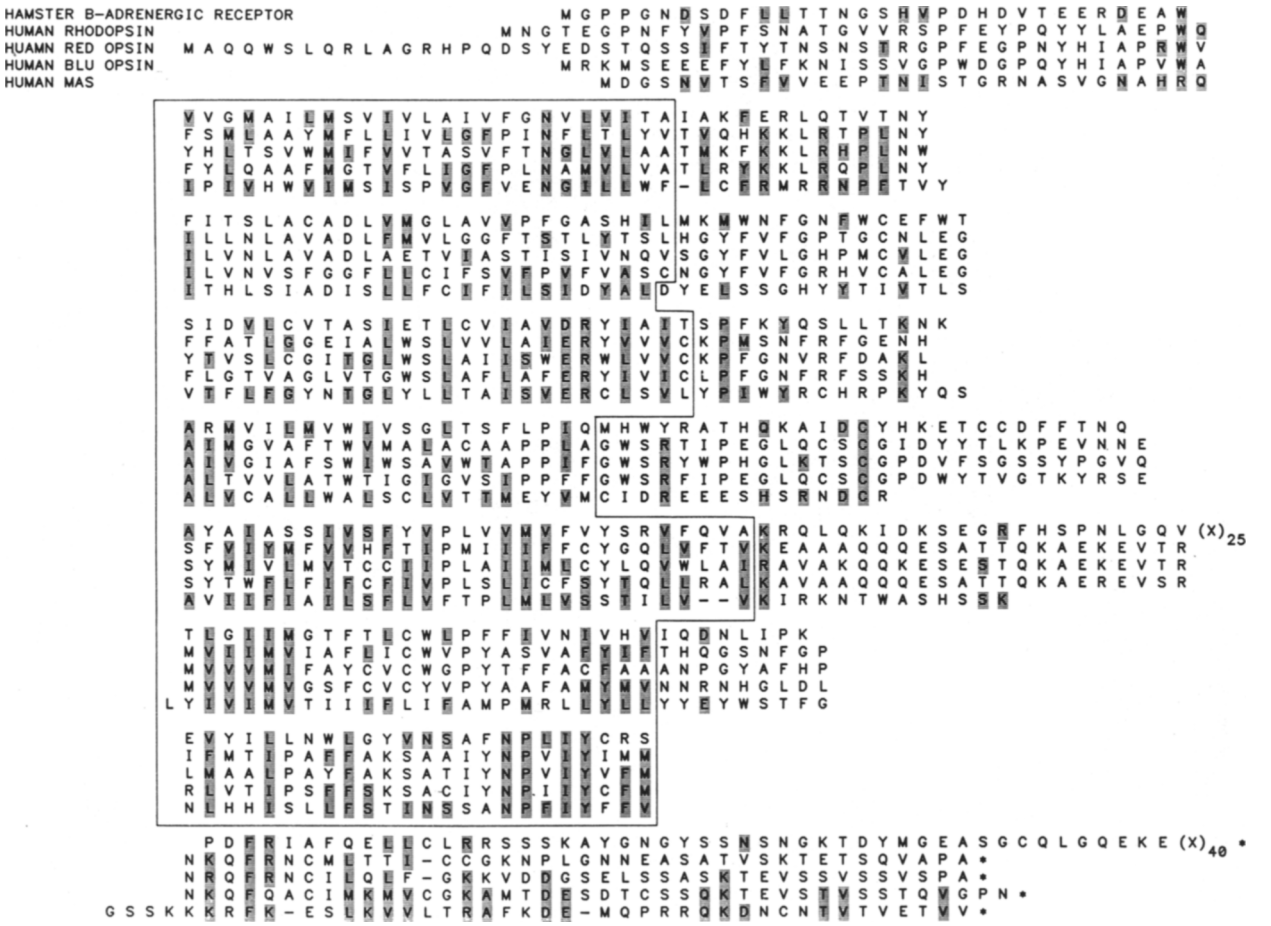

Figure 5. Comparison of the amino acid sequences of human *mas* protein, hamster β-adrenergic receptor (Dixon et al. 1986), human rhodopsin (Nathans and Hogness 1984), and the human visual color opsins (Nathans et al. 1986). Identical and similar amino acids are shaded. Similarity of amino acid residues was determined using values of Dayhoff et al. (1978).

guanine nucleotide-binding protein. The ligand of the β-adrenergic receptor is epinephrine, and the receptor is thought to act via the activation of intracellular guanine nucleotide-binding proteins as well. We speculate that *mas* may be a receptor that activates a guanine nucleotide-binding protein in response to binding a low-molecular-weight ligand. The unique nature of *mas* leads us to suspect that it may provide a new link in understanding growth control and signal transduction.

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