Isolation and Characterization of a New Cellular Oncogene Encoding a Protein with Multiple Potential Transmembrane Domains

Dallan Young, Gayle Waitches, Carmen Birchmeier, Ottavio Fasano,* and Michael Wigler
Cold Spring Harbor Laboratory
P.O. Box 100
Cold Spring Harbor, New York 11724

Summary

We have cloned and sequenced a new human oncogene and have named it mas. This oncogene was detected by its tumorigenicity in nude mice using the cotransfection and tumorigenicity assay previously described. The mas oncogene has a weak focus-inducing activity in transfected NIH 3T3 cells. A DNA rearrangement in the 5' noncoding sequence, which occurred during transfection, is probably responsible for activation of the mas gene. The cDNA sequence of the mas oncogene reveals a long open reading frame that codes for a 325 amino acid protein. This protein is very hydrophobic and has seven potential transmembrane domains. In this respect, the structure of the mas protein is novel among cellular oncogene products and may reflect a new functional class of oncogenes.

Introduction

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 possibly others (Goubin et al., 1983; Lane et al., 1984; Takahashi et al., 1985).

To search for oncogenes that may escape detection by the focus induction assay, our lab has explored a sensitive bioassay for transforming genes based on the tumorigenicity in nude mice of NIH 3T3 cells following cotransfection with a selectable marker and DNA from tumor cells. Using this assay, three transforming genes were derived from a human epidermoid carcinoma. This gene efficiently induces tumorigenicity and has a weak focus-inducing activity in NIH 3T3 cells. We have cloned cDNAs containing the entire coding sequence of mas. The mas gene encodes a protein with seven hydrophobic regions that are potential transmembrane domains, suggesting that mas is an integral membrane protein. The structure of mas protein is unique among cellular oncoproteins and may represent a new functional class.

Results

Isolation of the mas Oncogene

The mas oncogene was detected using the cotransfection and tumorigenicity assay we have previously described (Fasano et al., 1984). This method is a modification of the tumorigenicity assay described by Blair et al. (1982). DNA from a human epidermoid carcinoma was used to cotransfect NIH 3T3 cells with the plasmid pKOneo, and transfected cells were selected with the neomycin analogue G418. In one experiment, one of six nude mice injected with these cells developed a "primary" tumor within 4 weeks. Nude mice injected with cells cotransfected with DNA isolated from this primary tumor developed "secondary" tumors within 2 weeks. After a third round, DNA was purified from a "tertiary" tumor, and a genomic library was constructed in the cosmid vector pHC79. Four overlapping cosmids clones that contained human DNA were isolated by filter hybridization to total human genomic DNA (Gusella et al., 1980; Shih and Weinberg, 1982). Characterization of these cosmids by restriction mapping and Southern hybridization to total human DNA revealed that one of the cosmids, pMAS1, contains a 22 kb stretch of human DNA flanked by mouse DNA on both sides (Figure 1).

The pMAS1 cosmids was tested by the cotransfection and tumorigenicity assay to determine whether it contained transforming potential. Nude mice developed tumors within 2 weeks after injection of cells that had been cotransformed with pMAS1 and pKOneo and selected for resistance to G418 (see Table 1). Furthermore, in our standard focus assay, NIH 3T3 cells transfected with pMAS1 formed foci within 16 days. The foci of cells transformed with pMAS1 are unlike foci of cells transformed with the activated human H-rasV12 oncogene, isolated from the T24 bladder carcinoma cell line. They appear at a lower frequency about 4–6 days later (Table 2). The foci induced by pMAS1 are characterized not by an abnormal morphology of the constituent cells, but by an exceedingly high cell density (see Figure 2).

To define the regions of pMAS1 essential for transforming activity, various restriction endonuclease digests of pMAS1 were torted by the NIH 3T3 focus assay. When pMAS1 was cut with EcoRI or SalI, the transforming potential was destroyed, indicating that regions including one or more of each of these sites are essential for transforming activity (Table 2). In contrast, pMAS1 DNA cut with SmaI or XhoI retained its transforming ability. A 7.3 kb re-
region of pMAS1, which is defined by Smal and Xhol sites and contains single EcoRl and Sall sites, is capable of transforming NIH 3T3 cells. This region was subcloned into pUC8 to generate pMS422 (Figure 1).

Activation of the mas Oncogene by Rearrangement

Comparison of Southern blots of normal human DNA with DNA derived from the tertiary nude mouse tumor revealed a difference in the size and intensity of EcoRl restriction fragments homologous to mas (Figure 3), suggesting that the mas gene was rearranged and amplified in transfectants. To determine the nature of the DNA rearrangement, clones containing homology to a region of pMAS1 were isolated from a human placenta cosmid library. The probe used was the 2.2 kb Sall-EcoRI restriction fragment, which does not contain Alu-repetitive sequences and includes the 3' portion of the mas coding sequence (see next section). Three independent overlapping clones designated pHM1, pHM2, and pHM3 were obtained. Comparison of maps of restriction endonuclease sites in pHM2 and pMAS1 confirm that there is a break in homology localized between the EcoRl and Hpal sites in the 5' non-coding region of the mas gene (Figure 1). Although this break point is not in the mas coding region (see next section), it does occur in a region essential for transformation of NIH 3T3 cells by the mas gene (see above and Table 2). This suggests that the rearrangement found in the transformant is of functional significance.

The cosmid pHM2, which contains the normal human homolog of mas, was tested to determine whether it has transforming activity. By our standard transfection assay, pHM2 did not induce foci of transformed NIH 3T3 cells even after 4 weeks. However, pHM2 did induce tumors in a cotransfection and tumorigenicity assay, although with a longer lag time than pMAS1 (see Table 1). Therefore, the normal mas clone, pHM2, has a weak biological transforming activity detected by our tumorigenicity assays.

To test whether the DNA rearrangement was responsible for activation of mas transforming potential, we constructed the hybrid clone pGW34, which contains the 5' non-coding region of the rearranged gene and the entire coding region from the normal human homolog (see Figure 1 and next section). This hybrid clone had focus-inducing activity similar to the mas clone, pMS422 (Table 2). In contrast, pHM84, a subclone of the normal placenta

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Table 1. Tumorigenicity Assays

<table>
<thead>
<tr>
<th>Test Plasmid</th>
<th>Mean Tumor Diameter (mm) after Week:</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>pMAS-1</td>
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</tr>
<tr>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>pHM-2</td>
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Tumorigenicity assays were performed as previously described (Fasano et al., 1984). NIH 3T3 cells (8 x 10⁵ cells per plate) were cotransfected with 200 ng pKneo, 30 μg of high molecular weight NIH 3T3 DNA, and test plasmid (300 ng of pMAS1, 300 ng of pHM2, or 50 ng of pT24) per plate. Following transfection, cells were split 1:5 and were selected for resistance to the antibiotics G418. After 2 weeks, the cells were confluent and were injected into nude mice (10⁶ cells per mouse). Tumor formation at the site of injection was followed for 6 weeks, and the mean tumor diameter was measured. Each line in the above table represents a single animal injected with independently cotransfected pools of cells.
Table 2. Focus Assays

<table>
<thead>
<tr>
<th>Experiment</th>
<th>DNA ng/Plate</th>
<th>Foci/ng</th>
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<tr>
<td><strong>A</strong></td>
<td>pMAS1</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>pH2M2</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>pH2M2</td>
<td>1000</td>
</tr>
<tr>
<td></td>
<td>pT24</td>
<td>50</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>pMAS1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>pMAS1/Smal</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>pMAS1/Hpal</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>pMAS1/EcorI</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>pMAS1/Sall</td>
<td>100</td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>pMS422</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>pMS424</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>pGW34</td>
<td>400</td>
</tr>
<tr>
<td></td>
<td>pMS422</td>
<td>400</td>
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Focus assays in NIH 3T3 cells were performed as previously described (Penucho et al., 1981). NIH 3T3 cells were transformed with the indicated amount of test DNAs, either cleaved with the indicated restriction enzymes (Experiment B), or uncleaved (Experiment A and Experiment C). High molecular weight NIH 3T3 DNA (30 μg/plate) was used as carrier DNA. After the indicated times, the number of foci were scored, and the number of foci per nanogram of test DNA was calculated. See Figure 1 for a description of the plasmid clones.

Foci scored after 2 weeks.
Foci scored after 3 weeks.

clono (Figure 1), did not induce foci, suggesting that the DNA rearrangement is responsible for activation of mas transforming potential. Since this rearrangement lies outside of coding sequences, we suspect that activation of the mas gene results from overproduction or inappropriate production of its normal product. However, in order to make a definitive conclusion we need to know the nature of the normal gene transcript.

Finally, we sought to determine whether the mas gene was activated in the donor tumor DNA or, rather, became activated during cotransfection into NIH 3T3 cells. Southern blotting experiments demonstrated that the mas genes resident in the original human epidermoid carcroma did not have the rearranged structure found in the NIH 3T3 cotransformant, nor were they amplified (Figure 3). Moreover, we have not observed the transfer of the mas gene from the original tumor DNA in other cotransformants. Thus, we have no evidence that the mas gene was active in the original human tumor. Rather, it is likely that the mas gene became rearranged and activated during gene transfer from the original human tumor DNA.

Organization and Sequence of the mas Gene Transcript

To define the transcription unit and coding potential of the mas gene, we cloned cDNAs complementary to mas mRNA. Poly(A)<sup>+</sup> mRNA was purified from a cell line, MAS 133, derived from the nude mouse tumor from which pMAS1 was isolated. Blot hybridization of this RNA with the mas gene insert from pMS422 revealed a homologous mRNA approximately 2.5 kb in length (data not shown). A cDNA library was constructed from this poly(A)<sup>+</sup> RNA in the λgt11 vector and was screened for homology to pMS422. Sixteen overlapping cDNA clones were isolated and characterized by restriction endonuclease site mapping and DNA sequencing (see Figure 4). From sequence data we assembled a composite nucleotide sequence that contains a complete open reading frame of 975 bp (Figure 5). 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, pMC130. From these data we have assumed the orientation of transcription as shown in Figure 4. To demonstrate that the mas transforming product is coded by pMC130, we constructed a plasmid, pKC30, containing the coding region of pMC130 linked to the SV40 promoter. NIH 3T3 cells cotransfected with pKC30 and pKOneo and selected with G418 are tumorigenic in nude mice and give rise to foci (data not shown).

Comparison of the restriction endonuclease cleavage sites of genomic and cDNA sequences indicated that the cDNA is entirely colinear with genomic sequences. This observation was subsequently confirmed for the coding region by direct nucleotide sequencing of the homologous...
Figure 3. Southern Blot of Human and mas Transformant DNA
EcoRI-cut DNAs derived from: lane a, MAS-133 cell line; lane b, the
original human epidermoid carcinoma, and lane c, human placenta.
Blots were hybridized to the 2.2 kb SalI–EcoRI alu-repeat free frag-
ment of pMAS1. Open and closed arrows indicate positions of mas-
related sequences in MAS-133 and human genomic DNAs, respec-
tively. The molecular weights of size markers are indicated in kilobases
on the right.

region of the genomic DNA clone pMS422 (see Figure 5).
By S1 analysis we determined that the colinearity of the
mRNA with the genomic DNA extends in the 5' direction
approximately 45 bases beyond the first ATG in the coding
region (Figure 6). The site marking the beginning of
colinearity between RNA and genomic sequences may re-
fect a transcription initiation site or a splice site. Since no
cDNA clones containing poly(A) were found, it is likely that
mas transcripts extend beyond the 3' end of the cDNA
clone pMC142 (see Figure 4).

To learn more about the organization of the normal mas
gene and the events that can lead to its activation, we ex-
amined RNA in cells that were cotransformed with the
cosmid pHM2, selected for tumorigenicity in nude mice,
and subsequently placed back into culture. Total RNA was
prepared from these cells and was analyzed in the man-
ner described for MAS-133 cells (Figure 6). This analysis
indicates that transcripts of mas in these cells are also
colinear with genomic DNA 5' to the open reading frame
already identified. Hence, the protein encoded in these
cells is probably the same as in MAS-133.

Predicted Primary and Secondary Structure
of the mas Protein
The complete nucleotide sequence of the coding region
determined from mas cDNAs and the corresponding
predicted amino acid sequence of 325 amino acids are
shown in Figure 5. The deduced amino acid sequence
does not share significant homology with any published
sequence. Analysis of the hydrophobicity plot of the se-
quence by the method of Kyte and Doolittle (1962) reveals
that the mas protein has seven distinct hydrophobic
regions (Figure 7). Computational methods based on
hydrophobicity profiles have been developed to distin-
guish transmembrane regions from hydrophobic internal
regions of globular proteins (for review see Eisenberg,
1984). A recently developed algorithm correctly predicts
membrane spanning segments in many proteins, includ-
ing the seven segments in bacteriorhodopsin, and cor-
rectly disqualifies hydrophobic regions from many soluble
proteins (Eisenberg et al., 1984). This algorithm predicts
seven transmembrane domains in the mas protein corre-
sponding to the seven hydrophobic regions. Each of the
hydrophobic regions is separated by hydrophilic regions
that contain a predicted β-turn secondary structure (Chou
The Human \( \text{mas} \) Oncogene

Figure 5. Nucleotide Sequence of Coding and Flanking Regions of the \( \text{mas} \) Gene

The DNA sequence was derived from cDNA clones and the genomic subclone pMS422. The amino acid sequence deduced from the coding region is shown above the DNA sequence. The in-frame stop codons of the open reading frame are indicated by asterisks. The DNA sequence from the fourteenth nucleotide position 5' from the start AlG to the 3' end was derived from the cDNA clones. The sequence of the coding and 5' regions were determined from the genomic clone pMS422. The numbers on the right are amino acid coordinates.

and Fasman, 1978). Both the amino- and carboxy-termini of the \( \text{mas} \) protein are hydrophilic. This analysis strongly suggests that the \( \text{mas} \) protein is an integral membrane protein with many transmembrane domains.

Several proteins that span the membrane multiple times have been identified and studied. These include bacteriorhodopsin and the eukaryotic visual rhodopsins (Ovchinnikov, 1982), lactose permease (Foster et al., 1983), the acetylcholine receptor (Ross et al., 1977; F'opot and Changeux, 1984), the sodium ion channel (Noda et al., 1984), the \( (\text{Na}^+ \text{ K}^+)\text{ATPase} \) (Kawakami et al., 1985; Shull et al., 1985), the \( \text{Ca}^{2+}\text{ATPase} \) (MacLennan et al., 1985), and the erythrocyte anion exchange protein (Jay and Cantley, 1988). In Figure 7 we show the hydrophobicity plots of bovine rhodopsin and the \( \alpha \)-subunit of acetylcholine receptor for comparison with \( \text{mas} \) protein. There is a striking similarity in the hydrophobicity patterns of \( \text{mas} \) and rhodopsin, which may reflect structural and functional similarities in these proteins.

In Figure 8 we illustrate the relative positions of amino acids in the transmembrane domains of the \( \text{mas} \) protein, assuming an \( \alpha \)-helical structure. This figure shows the hydrophobic and hydrophilic domains can be clearly seen. This protein has a total of 28 positively charged and 21 negatively charged residues distributed primarily in the hydrophilic regions; however, there are some charged residues within the hydrophobic domains. Charged residues have been observed in many of the transmembrane domains of membrane proteins.

The \( \text{mas} \) protein does not contain an amino-terminal hydrophobic signal sequence characteristic of many membrane proteins (see Wickner and Lodish, 1985, for review on protein translocation). However, some proteins with multiple transmembrane domains, such as bovine rhodopsin, the erythrocyte anion exchange protein, and the sodium channel protein, lack amino-terminal signal sequences. In the first two cases, insertion into the membrane is cotranslational and requires internal signal sequences (Braell and Lodish, 1982; Friedlander and Blobel, 1985). Since the \( \text{mas} \) protein does not contain an amino-terminal hydrophobic signal sequence, its integration into the membrane may also depend on internal signal sequences, or alternatively, it may spontaneously become inserted into the membrane because of its hydrophobic nature (Blobel, 1980; Engelman and Steitz, 1981).

It has been established that the tripeptide sequence Asn-X-Thr/Ser is a site for N-glycosylation in secreted and membrane proteins (reviewed by Kornfeld and Kornfeld, 1985), although not all of these sequences are glycosylated (Struck and Lennarz, 1980). There are four potential sites for N-glycosylation in the predicted protein sequence of \( \text{mas} \) at positions 5, 18, 22, and 272. The first three of these sites are clustered in the first hydrophilic domain, while the fourth site is in the seventh hydrophilic domain.
Figure 6. S1 Analysis of RNA Transcripts

The Accl fragment of the genomic clone pMS422 was end-labeled, hybridized to RNA, and digested with S1 nuclease. RNA used was isolated from: lane d, NIH 3T3 cells; lane c, MAS-133; or lane b, NIH 3T3 cells transformed with the normal mas clone pHM2. This figure also shows: lane a, the 211 bp Stul-Accl fragment of pMS422; and lane e, molecular weight markers. Restriction endonuclease sites are indicated as follows: A, Accl; St, Stul; X, Xhol; and E, EcoRI. The Stul site is 10 bp 5' to the ATG initiation codon and includes the last base pair of the first in-frame stop codon. These data indicate that colinearity between RNA transcripts and genomic sequences from the mas gene extend 5' beyond the termination codon at the Stul site.

Discussion

The cotransfection and tumorigenicity assay was developed to search for transforming genes from tumor cells that may not be readily detected by the standard focus assay. Using this assay, three transforming genes were previously isolated from the MCF-7 cell line (Fasano et al., 1984). One of these genes is a normal N-ras gene, which is amplified in MCF-7 DNA. Another, designated mcf2, is currently being investigated, and the third gene, mcf3, is the human homolog of v-ras (Birchmeier et al., unpublished). In the latter case, the human ras gene was rearranged during or after gene transfer, probably resulting in its activation. A similar event appears to have occurred in the activation of the mas gene. The mas gene was found to be rearranged and amplified in the tertiary nude mouse tumor DNA. In contrast, the mas genes of the original human tumor DNA, used in the first round of cotransfection experiments, have the normal configuration and are not amplified. Although the normal human mas gene has weak tumor-inducing activity, it has no detectable focus-inducing activity. By contrast, the rearranged mas gene has strong tumor-inducing activity and can induce foci of NIH 3T3 cells. Analysis by chimeric gene construction indicates that a similar rearrangement can activate the normal placental allele. Therefore, we conclude that the cotransfection and tumorigenicity assay has a propensity...
The human mas oncogene

Figure 6. mas Protein Sequence

The hydrophobic domains are drawn assuming an α-helical structure with 3.6 residues per turn. The hydrophobic amino acids Leu, Ile, Val, Phe, Ala, and Met are shaded.

For detecting those proto-oncogenes that can induce tumorigenicity in NIH 3T3 cells by rearrangement and/or amplification following cotransfection. In this respect, the NIH 3T3 cotransfection and tumorigenicity assay differs considerably from the NIH 3T3 focus assay, which very rarely scores positive with DNA that does not already contain an activated oncogene. There is one reported case where the NIH 3T3 focus assay detected a transforming gene that may have been activated by a DNA rearrangement (Takahashi et al., 1985).

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' sequence with a sequence 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. However, we cannot rigorously exclude the possibility that the rearranged alleles have an altered splicing pattern, which results in an altered protein product. To resolve this problem completely, we must identify mas transcripts in a normal cell, which we have not yet been able to do. However, we have examined transcripts of the mas gene in cells cotransformed with the normal allele. S1 analysis indicates that in such cells we can exclude the existence of an additional amino-terminal coding domain.

There are currently about 30 known viral or cellular oncogenes; many code for proteins associated with the plasma membrane (Land et al., 1983). Three oncogenes, v-erbB, v-fms, and v-ros code for proteins with single transmembrane domains and are probably related to growth factor receptors (Downward et al., 1984; Hampe et al., 1984; Neckameyer and Wang, 1985). While ras and src do not encode transmembrane domains, they are associated with the membrane and have fatty acid residues that are added to the proteins by a posttranslational modification.
tyrosine phosphorylation and cellular transformation, activities that have been implicated in transformation (Hunter and Cooper, 1986). Although no direct proof links tyrosine phosphorylation and cellular transformation, a good correlation exists between the transforming abilities and tyrosine phosphorylation activities of viral transforming proteins.

In contrast to the structure of the membrane-associated oncogene products discussed above, the structure of the mas gene product implied from the cDNA sequence is very different. A computer search through the Protein Identification Resource and GENBANK data bases (see Experimental Procedures) found no significant homology between mas and any known DNA or protein sequences, including protein kinases or ras proteins. The hydrophobicity plot of the amino acid sequence indicates that there are seven very hydrophobic regions that are potential transmembrane domains. This strongly suggests that the mas protein is an integral membrane protein that may cross the plasma membrane many times. Only one transforming gene has previously been described that encodes a protein with multiple transmembrane domains. This gene, encoded by the Epstein-Barr virus, codes for a plasma membrane protein (LMP) with six potential transmembrane domains (Fennwahld et al., 1984) and has recently been shown to be capable of transforming Rat-1 cells (Wang et al., 1985). Unlike the mas protein, LMP has a long carboxy-terminal hydrophilic region. Moreover, unlike the mas gene, the LMP gene does not render NIH 3T3 cells tumorigenic.

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 the acetylcholine receptor and the visual rhodopsins. The acetylcholine receptor functions as a hormonally regulated ion channel. Visual rhodopsin is a light receptor that functions to activate transducin, an intracellular guanine nucleotide binding protein. We speculate that mas may be a receptor that activates a critical component in a growth regulatory pathway, perhaps by serving in signal transduction or as a membrane channel. The unique nature of mas leads us to suspect that it may provide a new link in understanding growth control.

Experimental Procedures

Focus and Tumorigenicity Assays

High molecular weight DNA was purified from cell lines (Peruch et al., 1981) and solid tumors (Fasano et al., 1984) as described. DNA transfer into NIH 3T3 cells were performed by a modified calcium phosphate precipitation method (Wigler et al., 1979). Focus assays (Peruch et al., 1981) and tumorigenicity experiments (Fasano et al., 1984) were performed as previously described. The plasmids p824 (containing the activated human H-rasv12 gene) and plKoneo (containing a neomycin/G418 antibiotic resistance gene) were previously described (Fasano et al., 1984).

Construction of Libraries

Genomic libraries were constructed in the cosmid vector pHC79 (Hohn and Collins, 1980; Maniatis et al., 1982) from EcoRI partially cleaved DNA and were screened by colony filter hybridization (Hanahan and Meselson, 1983). A cDNA library was constructed in λgt10 (Huynh et al., 1985) from purified poly(A)+ mRNA (Maniatis et al., 1982) from the MAS-133 cell line. The cDNA library was screened by plaque hybridization (Woo, 1979).

DNA Analysis

Southern blots were performed as previously described (Shimizu et al., 1983). S1 mapping was done by a modification of the Berk-Sharp method (Weaver and Weissman, 1979). DNA sequences were determined in both orientations by the dideoxy method of Sanger et al. (1977) as modified by Biggins et al. (1983). DNA and protein homology searches were performed using a previously developed algorithm (Goud and Kanehisa, 1982), and the Protein Identification Resource (National Biomedical Research Foundation, Georgetown University Medical Center, Washington, D.C.) and GENBANK (Boo, Beranek and Newman Inc., Cambridge, MA) data banks.

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