

## Cloning of the Complete Gene for Carcinoembryonic Antigen: Analysis of Its Promoter Indicates a Region Conveying Cell Type-Specific Expression

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Carcinoembryonic antigen (CEA) is a widely used tumor marker, especially in the surveillance of colonic cancer patients. Although CEA is also present in some normal tissues, it is apparently expressed at higher levels in tumorous tissues than in corresponding normal tissues. As a first step toward analyzing the regulation of expression of CEA at the transcriptional level, we have isolated and characterized a cosmid clone (cosCEA1), which contains the entire coding region of the CEA gene. A close correlation exists between the exon and deduced immunoglobulin-like domain borders. We have determined a cluster of transcriptional starts for CEA and the closely related nonspecific cross-reacting antigen (NCA) gene and have sequenced their putative promoters. Regions of sequence homology are found as far as approximately 500 nucleotides upstream from the translational starts of these genes, but farther upstream they diverge completely. In both cases we were unable to find classic TATA or CAAT boxes at their expected positions. To characterize the CEA and NCA promoters, we carried out transient transfection assays with promoter-indicator gene constructs in the CEA-producing adenocarcinoma cell line SW403, as well as in nonproducing HeLa cells. A CEA gene promoter construct, containing approximately 400 nucleotides upstream from the translational start, showed nine times higher activity in the SW403 than in the HeLa cell line. This indicates that *cis*-acting sequences which convey cell type-specific expression of the CEA gene are contained within this region.

The carcinoembryonic antigen (CEA) was originally described as a glycoprotein molecule with an oncofetal expression pattern (13). Recent experiments indicate that CEA may function as a cell adhesion molecule, which could play an important role during embryogenesis and possibly also during tumor development (5). Despite its presence in some normal tissues, its concentration in serum is a clinically useful parameter, especially in the postoperative monitoring of colonic tumor patients (52). CEA is a member of a family of closely related molecules, whose genes reveal a high degree of sequence similarity (reviewed in reference 49). The CEA family shows structural resemblance to, and can be placed within, the immunoglobulin superfamily (4, 32, 37). The CEA family members are made up of one N-terminal immunoglobulin variable-like, and a varying number of immunoglobulin constant-like domains, according to the classification of Williams (57). The CEA gene family can be divided into two main subgroups based on sequence comparisons. One subgroup contains the CEA gene itself (2, 4, 36, 59) and those encoding the classic CEA immunocross-reacting antigens, such as the nonspecific cross-reacting antigens (NCA) (2, 31, 35, 47, 51) and biliary glycoprotein (3, 17). These molecules are membrane bound, either as integral proteins such as biliary glycoprotein or after posttranslational modification through a covalently linked glycosyl phosphatidylinositol moiety as shown for CEA (16, 19, 45) and an NCA (15, 21). The second subgroup contains the genes encoding the pregnancy-specific glycoproteins (PSG), which were recently shown to have homology to the CEA subgroup members (56). These proteins are expressed at

high levels in the placenta (26) and at lower levels in trophoblastic tumors (46).

Despite the high sequence similarity of CEA and PSG, they show differential expression patterns. PSG mRNAs are found in the placenta, where no apparent expression of any of the CEA gene subgroup mRNAs can be determined (48, 61). Colonic tumors, on the other hand, contain transcripts for CEA and NCA but not PSGs (48, 60, 61). In addition, differential expression of CEA and NCA mRNAs can be found in various other tumors (7, 60). There is a notable difference in the amounts of CEA protein found in normal colonic mucosa compared with colonic tumors (8). Some evidence has recently been presented which would suggest that a rapid loss of CEA takes place in normal colonic mucosa, but not in the corresponding tumors, and that the rates of synthesis are comparable in both cases, which would explain these differences (23). However, other results suggest an increased transcriptional activity in colonic tumors compared with the normal mucosa (7), which would also suggest regulation of expression at the transcriptional level. Indeed, differential methylation patterns could be shown to correlate with the rate of CEA expression in various cell lines (54). Recently, other factors have been reported to exist which also play a role in the regulation of transcription and translation of CEA-related genes (53).

As a basis for more detailed studies on the transcriptional regulation of tissue-specific and developmental expression of CEA, as well as its possible role during embryo and tumor development, it is necessary to characterize the CEA gene. In this paper, we report the isolation of a cosmid clone which contains the complete transcriptional unit of the CEA gene, including its promoter region. We have carried out restriction endonuclease mapping and have determined the exon

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structure of this gene. Sequence and functional analyses of the putative promoter regions of the CEA gene, together with an NCA gene whose isolation we have described elsewhere (51), have been carried out to locate their promoters. Furthermore, we have identified a region which apparently conveys tissue-specific expression of the CEA gene.

## MATERIALS AND METHODS

**Chemicals and oligonucleotides.** Enzymes were purchased from Boehringer Mannheim Biochemicals, Bethesda Research Laboratories, Inc., and Pharmacia/LKB. Radiochemicals, RNasin, and Taq polymerase were from Amersham Corp. and Du Pont, NEN Research Products. All other chemicals were of analytical grade. Oligonucleotides were synthesized by the phosphoramidite method on an Applied Biosystems 308A DNA synthesizer and purified by following the manufacturer's protocol, as described previously (20).

**Bacterial strains and cloning vectors.** All work with recombinant DNA was carried out in accordance with the German and U.S. safety regulations. A human cosmid genomic library inserted in the vector pHC79-2cos/tk (27) was obtained as packaged cosmids from W. Lindenmaier, Gesellschaft für Biotechnologische Forschung, Brunswick, Federal Republic of Germany, and transduced into *Escherichia coli* ED8767 by combining 0.1 ml of 50 mM Tris hydrochloride (pH 7.5), 10 mM MgSO<sub>4</sub>, 0.1 ml of ED8767 in LB medium (optical density at 600 nm = 2) and 0.1 ml of packaged cosmids. After incubation for 30 min at 30°C the suspension was diluted with 2 ml of LB medium, incubated for 60 min at 37°C with shaking, and plated out. For subcloning of the insert DNA fragments, either the Bluescript phagemid (Stratagene Inc.) and pUC18/19 were used in *E. coli* JM109 and RR1ΔM15, or M13mp18/19 bacteriophage DNA was transfected and amplified in *E. coli* JM107.

**Screening of the human genomic cosmid library.** Recombinant clones were grown on nitrocellulose (Schleicher & Schuell, Inc.) in the presence of ampicillin (50 μg/ml) and amplified after the addition of chloramphenicol (150 μg/ml). Colony hybridization with a CEA-specific, <sup>32</sup>P-labeled, 408-base-pair (bp) *RsaI-PstI* restriction endonuclease fragment from the 3' untranslated region of a CEA cDNA clone (pCEA3 [60]) was performed in the presence of 50% formamide, 6× SSPE (1× SSPE is 0.18 M NaCl, 10 mM NaH<sub>2</sub>PO<sub>4</sub> [pH 7.4], and 1 mM EDTA), 1× Denhardt solution (0.02% each Ficoll, polyvinylpyrrolidone, and bovine serum albumin), 0.05% sodium phosphate, and 100 μg of sheared, denatured salmon sperm DNA per ml at 42°C. The final wash was in 2× SSPE–0.5% sodium dodecyl sulfate at 65°C.

Positive clones were purified and, for identification of a full-length CEA gene clone, were hybridized with a <sup>32</sup>P-labeled synthetic oligonucleotide (CEAL, 5'-AGCCTCTGC CAGGGGATGCACCAT-3') corresponding to nucleotide positions 1798 to 1821 of the leader region of the CEA gene (see Fig. 2). The hybridization took place in 50% formamide–1 M NaCl–1% sodium dodecyl sulfate–100 μg of sheared, denatured salmon sperm DNA per ml–10% dextran sulfate at 30°C, and washing was carried out in 2× SSPE–1% sodium dodecyl sulfate at 10°C below the calculated melting temperature (24).

**Restriction endonuclease mapping.** The cosmid clone was mapped by digestion with the restriction endonuclease *AatII*, which has three recognition sites in the vector. A 32.6-kilobase-pair (kb) *AatII* fragment contains the whole of the insert and flanking vector sequences (0.1 and 1 kb). This fragment was then partially digested with different restric-

tion endonucleases (*BamHI*, *SaII*, *SmaI*, *SstI*, and *XbaI*), separated by gel electrophoresis in 0.5% agarose overnight at 1 V/cm (4°C), and transferred to GeneScreen Plus membrane (Du Pont, NEN) prior to hybridization with an oligonucleotide probe, whose complementary sequence is located in the 0.1-kb flanking sequence (cos1, 5'-TAGGCGTAT CACGAGGCCCTTTTCG-3'). After stripping, the blot was rehybridized with an oligonucleotide (cos2, 5'-GGCGATGC TGTCGGAATGGACG-3') whose corresponding sequence is located in the 1-kb flanking sequence. After autoradiography, the linear arrangement of restriction sites in the partial digests was determined from the fixed *AatII* sites. Uncertainties arising from this method could be resolved by isolation of selected DNA fragments, followed by digestion with a second restriction endonuclease and by agarose gel electrophoresis separation.

The N-terminal domain and 5' untranslated regions were located by Southern blot analysis with the 547-bp *PstI* fragment from pNCA1 (20) and the CEAL oligonucleotide (see above) from the leader region of the CEA cDNA, respectively, as probes. The exon/intron boundary-containing fragments were located on Southern blots by using oligonucleotide probes homologous to regions at the beginning and end of each A and B immunoglobulin constant-like domains. The cosCEA1 DNA was digested with various restriction endonucleases and transferred to Zeta-probe membrane (Bio-Rad Laboratories) as suggested by the manufacturer. The membranes were hybridized with the various oligonucleotides in 5× SSC (1× SSC is 0.015 M sodium citrate plus 0.15 M NaCl)–0.5% nonfat dry milk–0.01% sodium dodecyl sulfate. The hybridization and wash temperatures were calculated by the method of Wallace and Miyada (55). The CEA 3' untranslated region was located by using an approximately 450-bp *RsaI-EcoRI* cDNA fragment downstream of the first Alu sequence (36).

**Determination of DNA sequences.** Subcloned restriction endonuclease DNA fragments in phage M13mp18/19, pUC18/19, and Bluescript phagemid vectors were sequenced as single- or double-stranded templates by the dideoxy-chain termination method (40), with universal or internal oligonucleotide primers and a kit from United States Biochemical Corp. For comparison of the nucleotide and deduced amino acid sequences, the computer program Align (M. Trippel and R. Friedrich, unpublished data) was used. The DNA sequence data have been forwarded to GenBank and have received the accession numbers M31966 to M31975.

**Determination of the transcriptional starts of the CEA and NCA genes.** (i) **Primer extension experiments.** Gene-specific oligonucleotides complementary to CEA and NCA mRNA species were used to prime extension reactions. We synthesized oligonucleotides from the leader region of the CEA gene (CEAL, see above) and the 5' untranslated region of the NCA gene (NCA5'; 5'-TCTCTGTCCACCTCTTTG TAGAGGA-3', corresponding to positions –15 to –39 in Fig. 5). For the annealing reaction, 5 to 10 ng of oligonucleotide labeled with <sup>32</sup>P at the 5' end was mixed with 2.5 μg of poly(A)<sup>+</sup> RNA isolated from a colon tumor (59) in 10 μl of 5 mM sodium phosphate buffer (pH 7.0)–5 mM EDTA. After a 5-min denaturation at 90°C, NaCl was added (final concentration, 80 mM), and hybridization took place at 50°C for 1 h. After this time the mixtures were allowed to cool slowly to room temperature. The hybridization mix was adjusted to 17.5 mM Tris hydrochloride (pH 8.3), 4.3 mM MgCl<sub>2</sub>, 1.75 mM dithiothreitol, 3.5 mM deoxyribonucleoside triphosphates, 1 ng of dactinomycin per μl, 2 U of RNasin per μl, and 0.8 U of reverse transcriptase per μl in a volume of 25 μl

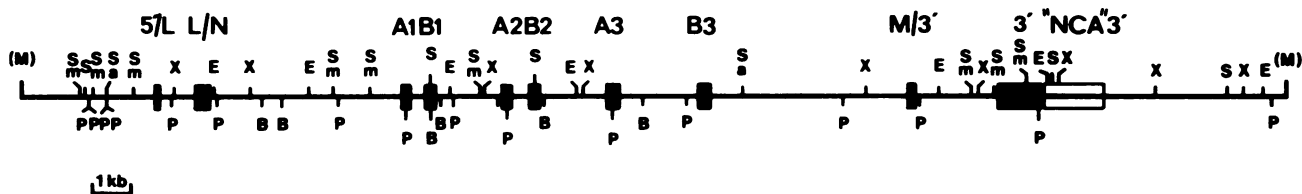


FIG. 1. Restriction map of cosCEA1. The following restriction endonuclease sites have been mapped: *Bam*HI (B), *Eco*RI (E), *Pst*I (P), *Sai*I (Sa), *Sma*I (Sm), *Sst*I (S), *Xba*I (X), and degenerate *Msp*I cloning sites [(M)]. Symbols: ■, exons 5/L through 3'; □, "NCA"3' (this exon has been only provisionally characterized; see text). The exon names shown above the map are explained in the text.

and incubated at 42°C for 1 h. Following phenol extraction and ethanol precipitation, the extension products were de-natured and loaded onto a 6% polyacrylamide-7 M urea DNA sequencing gel.

(ii) **S1 nuclease analyses.** The transcriptional starts of CEA and NCA were independently determined by S1 nuclease mapping. For CEA, a 1.6-kb *Pst*I restriction endonuclease fragment from cosCEA1, containing the first exon of the CEA gene (Fig. 1), and for NCA a 606-bp *Pst*I fragment from clone lambda 39.2 (51), containing the first exon of the NCA gene, were cloned into M13mp18. Gene-specific, single-stranded DNA fragments which were complementary to their mRNAs were synthesized by using the same oligonucleotides as for the primer extension experiments (see above). For this, the oligonucleotides were 5' end labeled with polynucleotide kinase and annealed to the single-stranded M13 subclones by the Perkin Elmer Cetus GeneAmp DNA amplification protocol, followed by extension with Taq polymerase at 72°C for 30 min. The resulting double-stranded products were digested with *Sma*I (for CEA) or *Pst*I (for NCA), and the single-stranded 456nc CEA-specific fragments and the 562nc NCA-specific fragments were separated on a preparative 4% polyacrylamide-7 M urea gel and extracted by electroelution. Hybridization with poly(A)<sup>+</sup> RNA from a colonic tumor (see above) and S1 nuclease mapping were performed essentially as described by Maniatis et al. (29). For digestion of single strands, the DNA-RNA complexes were incubated with 1,000 U of S1 nuclease per ml for 30 min at 18°C and analyzed as above.

**Construction of promoter-CAT gene hybrids.** The 3.4-kb *Sst*I-*Eco*RI fragment of the cosmid clone cosCEA1 (Fig. 1), which contains the first two exons of the CEA gene, was digested with *Nco*I. The 835-bp *Nco*I fragment was isolated and treated with S1 nuclease to generate blunt ends, resulting in an 831-bp DNA fragment located at nucleotide positions -832 to -2 with respect to the translational start of the CEA gene. The vector pBLCAT3 (28), which contains the promoterless bacterial chloramphenicol acetyltransferase (CAT) gene was linearized by using *Bam*HI. The ends were filled in with the Klenow fragment of *E. coli* DNA polymerase I, and the blunt-ended *Nco*I fragment was inserted to generate the constructs pCEA832/2CAT and pCEA2/832CAT (see Fig. 6). A 409-bp *Pst*I-*Bst*XI fragment was removed from clone pCEA832/2CAT, and after generation of blunt ends with T4 DNA polymerase, the rest was religated, resulting in the construct pCEA424/2CAT (see Fig. 6). In parallel, a 420-bp *Hind*III-*Bst*XI fragment of plasmid pCEA832/2CAT was removed, and the rest was ligated to a 2.9-kb *Hind*III-*Bst*XI fragment from the 5' end of cosCEA1 (Fig. 1), generating the construct pCEA3300/2CAT (see Fig. 6).

The 2.7-kb *Eco*RI fragment from the genomic NCA clone lambda 39.2 (51), which contains the first and second exons of the NCA gene, was subcloned into pUC18. This was

digested with *Sst*I, which cuts in the polylinker of pUC18 and internally, generating a fragment containing the 5' untranslated region of the NCA gene, extending from nucleotide positions -48 to -1246 with respect to the translational start (see Fig. 5). This was treated with T4 DNA polymerase to generate blunt ends and inserted into the above-mentioned, blunt-ended pBLCAT3 vector. Clones were constructed containing the inserts in both orientations (pNCA1246/48CAT and pNCA48/1246CAT; see Fig. 6). The construct pNCA1246/48CAT was digested with *Pst*I, the 663-bp fragment from the 5' region of the insert was removed, and the rest was religated, resulting in the construct pNCA583/48CAT (see Fig. 6). A 299-bp *Hind*III fragment containing vector sequences and the 5' portion of the insert from clone pNCA583/48CAT was removed. Religation of the rest generated the construct pNCA284/48CAT (see Fig. 6).

**Cells and transfection.** The colon adenocarcinoma cell line SW403 (25) was grown in RPMI 1640 medium supplemented with 10% fetal calf serum. HeLa cells were cultivated in Dulbecco modified Eagle medium containing 10% fetal calf serum. Cells were plated at a density of 10<sup>6</sup> per 6-cm dish 24 h before transfection. The transfections were carried out by lipofection with 20 μl of Lipofectin (Bethesda Research Laboratories) and 7 μg of pSV2CAT (14) DNA or equimolar amounts of the promoter-CAT gene constructs in 3 ml of cell medium without serum, modified as described by Felgner et al. (12). These parameters were optimized for both cell lines. The cells were incubated for 15 h, and then 3 ml of medium plus 10% fetal calf serum was added; 24 h later the medium was replaced with fresh medium. After a further 24 h of incubation, the cells were harvested in phosphate-buffered saline-1 mM EDTA without bivalent cations, by using a rubber policeman.

**CAT assay.** Pelleted cells were suspended in 75 μl of 250 mM Tris hydrochloride (pH 7.5), and cell extracts were made by three freeze-thaw cycles. Thereafter, the cells were spun down for 10 min at 13,000 × *g* and 4°C. The supernatants were incubated for 10 min at 65°C, and the CAT activity of the cell extracts was analyzed as described previously (14). The assay mixture contained (in a final volume of 100 μl) 30 μl of cell extract, 20 μl of 8 mM chloramphenicol, 20 μl of 0.5 mM [<sup>14</sup>C]acetyl coenzyme A (7.4 kBq), and 30 μl of 250 mM Tris hydrochloride (pH 7.5) and was incubated for 1 h at 37°C. After two extractions with 100 μl of ice-cold ethyl acetate to separate the radio labeled acetyl coenzyme A, half of the organic phase containing radiolabeled acetylated chloramphenicol was analyzed by liquid scintillation counting in Aquasol (Du Pont, NEN).

## RESULTS

**Isolation and characterization of cosCEA1.** A CEA-specific cDNA restriction endonuclease fragment from the 3' untranslated region (see Materials and Methods) was used to

probe 340,000 cosmid clones from a human genomic library. Three positive clones were isolated whose restriction endonuclease and hybridization patterns with CEA cDNA and oligonucleotide probes from different regions of the gene revealed them to be identical (data not shown). Closer analysis of this clone (cosCEA1) by restriction endonuclease mapping (Fig. 1) and DNA sequence analysis (Fig. 2) revealed that the 31.5-kb insert contains 11 exons, 10 of which represent the complete sequence for the CEA mRNA. The first exon contains the 5' untranslated region (see below) and part of the leader domain (5'/L exon). The second exon encodes the rest of the leader and the complete N-terminal (immunoglobulin variable-like) domain (L/N exon). The following six exons encode immunoglobulin constant-like half repeats (A and B exons), revealing a strong correlation between the exon and domain borders. A closer analysis of the derived amino acid sequences shows that the immunoglobulin-like domains, which contain a predicted, highly ordered arrangement of  $\beta$ -sheets, are always surrounded by proline-rich regions in CEA. These regions would disrupt ordered structures, thus marking the boundaries between adjacent domains. Such proline-rich regions are located close to the exon borders (Fig. 3). The B3 exon is followed by two further exons: the first contains the hydrophobic membrane domain and part of the 3' untranslated region (M/3' exon), and the next contains the rest of the CEA 3'-untranslated region (3' exon).

**Determination of the transcriptional starts of the CEA and an NCA gene.** As a basis for closer studies regarding the regulation of transcription of the CEA gene, we have determined its transcriptional start through primer extension (Fig. 4A) and S1 nuclease analyses (Fig. 4B). In both assays, a tight cluster of transcriptional start sites was located 104 to 110 nucleotides upstream from the translational start of the CEA gene (Fig. 4). The primer extension assay indicates transcriptional start positions which are 2 or 3 nucleotides shorter than those determined by the S1 nuclease assay. This difference is probably due to premature termination of the reverse transcriptase in the former assay, presumably because of steric interference by the cap structure, as reported elsewhere for a different gene (18). The location of the transcriptional start site cluster is comparable to that found by Beauchemin et al. (4) for the CEA mRNA. These authors, using only the primer extension analysis determined the transcriptional start to be 102 nucleotides upstream from the translational start.

We have also mapped the transcriptional start for an NCA gene, whose isolation we reported elsewhere (51), by using primer extension and S1 nuclease analyses (Fig. 4). It could be shown that this gene also has a cluster of transcriptional start sites at a similar position to that found for the CEA gene (between nucleotide positions -102 and -112, relative to the translational start).

**Sequence comparison of the putative promoter regions with other CEA-related genes.** We have sequenced the region around and upstream from the first exon of the NCA

genomic clone (Fig. 5). The putative promoter and upstream region of a third gene, CGM1, which was published recently (50), is compared with the NCA sequence in Fig. 5. It is obvious that NCA and CGM1 are homologous over the whole region that was compared. Although no transcripts have so far been identified for the CGM1 gene, this homology indicates that we may expect a similar expression pattern as for NCA, assuming that this gene is active. In comparison, the CEA gene reveals homology in a region up to approximately 500 nucleotides upstream of the initiator codon, but no homology could be determined farther upstream by dot matrix analyses (data not shown).

No canonical TATA or CAAT boxes could be identified at the expected positions (10) upstream of the transcription initiation sites of the CEA or NCA genes. A TATA sequence, which was also described elsewhere (35), can be found approximately 130 nucleotides upstream from the transcriptional start of the NCA gene. However, this distance would appear to be too large to have functional importance, because in most eucaryotic genes TATA sequences are almost always located 25 to 30 bp upstream from the initiation site, except in yeasts, for which distances of up to 120 bp have been described (reviewed in reference 44). Indeed, this putative TATA box is missing in the CEA gene at the corresponding position (Fig. 5), although 2 nucleotides downstream a degenerate TATA sequence can be found (TAGAA).

**Analyses of the CEA and NCA gene promoters through transient-transfection assays.** To determine the exact locations of the CEA and NCA gene promoters, it was necessary to use a cell line in which the CEA and NCA genes are transcriptionally active. We chose the CEA-producing human adenocarcinoma cell line SW403 (25, 59) and, as a negative control, HeLa cells (59). Northern (RNA) blot analysis revealed transcripts for both CEA and NCA in SW403 cells, but not in HeLa cells (59; data not shown). Various restriction endonuclease fragments, containing up to 3,300 bp of the region upstream from the translational start of the CEA gene, or up to 1,246 bp of the region upstream of the NCA gene translational start, were attached to the bacterial CAT reporter gene. The activities of the various promoter constructs were tested in SW403 and HeLa cells. The relative strengths of the promoter fragments were determined by quantification of the CAT enzyme activity. For comparison, plasmid pSV2CAT, which contains the simian virus 40 early promoter in front of the CAT gene, was transfected into each cell line and CAT activity levels were measured. By using the assumption that the simian virus 40 promoter is equally active in both cell lines, the values obtained for each of the CEA and NCA promoter constructs were expressed as a percentage of the values obtained with pSV2CAT. All of the CEA and NCA constructs were active in their correct orientations, in both cell lines (Fig. 6). Apart from the pNCA1246/48CAT construct, for which the activity in HeLa cells was slightly higher than in SW403 cells, the other NCA constructs showed similar

FIG. 2. Nucleotide sequence of cosCEA1. Intron and exon nucleotides are shown in lowercase and capital letters, respectively. The exon boundaries are marked with arrows over the nucleotide sequences. Restriction endonuclease sites used in subcloning fragments for sequencing are indicated. Polyadenylation sites are underlined. The poly(dA-dC) sequence is heavily underlined. Alu-type repetitive sequences are underlined with dotted lines. The approximate size of nonsequenced regions are shown in brackets and are taken into account for the numbering system (indicated in the margin by an asterisk). The exons were sequenced through the exon/intron boundaries (ca. 100 nucleotides) to confirm identity with the published cDNA sequence of Oikawa et al. (36). In no cases were any substitutions found. A polypurine sequence was noted at the beginning of the gene (1516 to 1588).

<b>Set1</b>		<b>Pat1</b>		<b>Pat1</b>		<b>Pat1</b>		<b>Pat1</b>		
gagctcctca	caoygactct	gtcagctcct	ccctgcagcc	tatnggcocg	ccacctgagg	cttgtcggcc	gccacttga	ggcctgtcgg	ctgcctctg	100
gagggagctc	ctgtccoccta	caccocctcc	ttcccogggc	tcagctgaaa	ggggctctcc	cagggcagct	ccctgtgatc	tcaggagacag	ctcagctctct	200
caagayctcc	gacgcocccct	atgctgtcac	ctcacagccc	tgctattacc	atlaactcct	cagtcccatg	aaagtcaactg	agccgctgtc	tcccoggttac	300
aggaaaactc	tgtgacaggg	accaogtctg	tctgtctctc	tgtggaatcc	cagggcccag	ccagtgctcg	acacggaaca	gatgctccat	aaatactggt	400
taaatgtgtg	ggagatctct	aaaaagaaac	atatcacctc	cgtgtggccc	ccagcagtc	gagctgtctc	catgtggaca	caggggcact	ggcaccagca	500
								<b>Pat1</b>		
tgggaggagg	ccagcaagtg	cccgcggctg	cccaggaat	gaggcctcaa	ccccagagc	ttcagaaggg	aggacagagg	ccctgcagggg	atagatcctc	600
	<b>Pat1</b>									
cgogctgacc	ctgcagoccta	atcttgagtt	cagggctcagc	tcacaccaog	togacctgg	tcagcatccc	tagggcagtt	ccagacaagg	coggaggtct	700
cctcttgccc	tccagggggg	gacattgcac	acagacatca	ctcaggaaac	ggattccoct	ggacaggaac	ctggcttgc	taaggaagtg	gaggtggagc	800
ctggtttcca	tcocctgtct	caacagagcc	ttctgatctc	tcocacatac	ctgctctggt	cccttctggg	tcctctgagg	acotgttctg	ccaggggtcc	900
ctgtgcaact	ccagactccc	tctgtgtacc	accatgggga	aggtgggggtg	atcacaggac	agtcagocctc	gcagagacag	agaccacca	ggactgtcag	1000
ggagaacatg	gacagggcct	gagocgcagc	tcagccaaca	gacacggaga	gggagggctcc	ccctggagcc	ttcccacaag	acagcagagc	ccagagtca	1100
ccacctccct	ccacacagct	cctctctttc	caggacacac	aagacacctc	ccocctccaca	tgcaggatct	ggggactcct	gagacctctg	ggcctgggtc	1200
tcactccctg	ggtcagtgcc	ggggttggtg	gtactggaga	gtccctccc	gtccctccc	agccaccacc	cagtgagcct	ttttctagoc	ccagagocca	1300
cctctgtcac	cttctgtgtg	ggcatcatcc	caacctccca	gagccctgga	gagcatgggg	agaccocggga	cctgctgggt	ttctctgtca	caaaggaaaa	1400
taatccccct	ggtgtgacag	acccaaggac	agaacacagc	agaggtcagc	actggggaaa	gacaggttgt	ccacagggga	tgggggtcca	tcocacttgc	1500
cgaaaaagatt	tgtctgagga	actgaaaata	gaaggaaaa	aagaggaggg	acaaaagagg	cagaatgag	aggggagggg	acagaggaca	cctgaataaa	1600
					<b>5'UT</b>					
gaccacacc	atgaccacg	tgatgctgag	aagtactcct	gcocctagaa	gagactcaGG	GCAGAGGGAG	GAAGGACAGC	AGACCAGACA	GTCACAGCAG	1700
							<b>Leader</b>			
OCTTGACAAA	AGTTTOCTGG	AACTCAAGCT	CTTCTOCACA	GAGGAGGACA	GAGCAGACAG	CAGAGACCAT	GGAGTCTOCC	TGGGCOOCTC	CCACAGATG	1800
							Me tGluSerPro	SerAlaProP	roHisArgTr	
			<b>Intron 1</b>							
GTCATTOCC	TGGCAGAGGC	TOCTGCTCAC	AGgtgaaggg	aggacaaccc	ctgggagagg	gtgggaggg	ggagcacaga	gactggctg	ggtctcctg	1900
pCysIlePro	TrpGlnArgL	euLeuLeuTh	rA							
gtaggacag	gctgtgagac	ggacagaggg	ctcctgttgg	agcctgaata	gggaagagga	catcagagag	ggacagaggt	cacaccagaa	aaatcaaat	2000
gaactggaat	tggaaaaggg	caggaaaacc	tcaagagttc	tattttccta	gttaattgtc	actggccact	acgtttttaa	aaatcataat	aactcatca	2100
gatgacactt	taataaaaa	cataaacagg	gcatgaaca	ctgtcctcat	ccgcctacog	oggacattgg	aaaataagcc	ccaggctgtg	gagggccctg	2200
ggaacctca	tgaactcacc	cacaggaatc	tcagacctgt	ccagggcact	gggtgcaacc	aagatcacac	aaatccctgc	cctcatgaag	ctcatgctct	2300
catggggagg	aagacagaca	tacaagaga	tctagaatgt	gaggtcaggt	gttgacaaga	gcctggaggg	aatagagcag	ggaaaaggtc	gaaaaggaag	2400
accocaggtc	tctagaggag	gtgtcagggc	agggatctcc	caagaatgcc	ctgatgtgag	caggacctga	agccaatggg	gagggagccg	tgaagccoc	2500
tggaaaagca	gattocacac	agggaaatgc	caaggtogga	ggtgctaagg	aaataggaga	cacactgctg	accttgacct	agtaggacac	acacacacac	2600
acacacacac	actcactcac	tccagggctg	ggggatgaa	agacctgtc	aggaoccaag	accocatttt	tccaccctaa	tgcataggtc	ccaatattga	2700
		<b>Leader</b>				<b>N-domain</b>				
ccgatgctct	ctgctctctc	ctagcCTCAC	TTCTAACCTT	CTGGAACCOG	COCAOCCTG	OCAAGCTCAC	TATTGAATOC	ACGCOGITCA	ATGTGCGAGA	2800
		laSerL	euLeuThrPh	eTrpAsnPro	ProThrThrA	laLysLeuTh	rIleGluSer	ThrProPheA	snValAlaGl	
GGGAAGGAG	GTCCTTCTAC	TGTGTCACAA	TCTGCCOCAG	CATCTTTTGG	GCTACAGCTG	GTACAAAGGT	GAAAGAGTGG	ATGGCAACOG	TCAAATTAATA	2900
uGlyLysGlu	ValLeuLeuL	euValHisAs	nLeuProGln	HisLeuPheG	lyTyrSerTr	pTyrLysGly	GluArgValA	spGlyAsnAr	gGlnIleIle	
GGATATGTAA	TAGGAACCTA	ACAAGCTACC	CCAGGGCOOG	CATACAGCTG	TCGAGAGATA	ATATACOOCA	ATGCATCOCT	GCAATGATOC	AGCINATOC	3000
GlyTyrValI	leGlyThrGl	nGlnAlaThr	ProGlyProA	laTyrSerGl	yArgGluIle	IleTyrProA	snAlaSerLe	uLeuIleGln	AsnIleIleG	
								<b>Intron 2</b>		
AGAATGACAC	AGGATTCTAC	AOCTCACOG	TCATAAAGTC	AGATCTTGTG	AATGAAGAAG	CAACTGGCOA	GTTCOOGGTA	TACCGtgagt	gattccccca	3100
lnAsnAspTh	rglyPheTyr	ThrLeuHisV	alIleLysSe	rAspLeuVal	AsnGluGluA	laThrGlyGl	nPheArgVal	TyrP		
tgacctctgg	gtgttggggg	tcagttctac	ttccacaca	caggattatc	agccctgggc	tgtgctgtgg	ccocctctgc	attaogaacc	atgttagggt	3200
					<b>EcoRI</b>					
ttgggcattt	agtgaggat	acacacagaa	gagacaaact	tcaacagatc	agaattcctt	tcocgcctcc	agaccctgca	g..... (ca 4.6kb)...		7900*
tgttccagat	catogtgcac	ctgtctgtg	acacatgcac	ccacogtggg	xtttttaagg	gctcaggtgg	gctgagaggt	ggaaagtgcc	aactctgatt	8000
						<b>Al domain</b>				
gaaagatgoc	tgtgaggaat	caaaggtxca	cacagggxaa	tcttctctct	gttatctgca	agGGAGCTG	COCAAGCOCT	CCATCTOCAG	CAACAACCTC	8100
						roGluLeu	ProLysProS	erIleSerSe	rAsnAsnSer	
AAACCOGTG	AGGACAAGGA	TGCTGTGGOC	TTCACCTGTG	AACTGAGAC	TCAGGACGCA	ACCTACCTGT	GGTGGGTAAA	CAATCAGAGC	CTCOOGTCA	8200
LysProValG	luAspLysAs	pAlaValAla	PheThrCysG	luProGluTh	rGlnAspAla	ThrTyrLeuT	rpTrpValAs	nAsnGlnSer	LeuProValS	
GTOCCAGGCT	GCAGCTGTCC	AATGGCAACA	GGACCOCTCAC	TCTATTCAT	GTCACAAGAA	ATGACACAGC	AAGCTACAAA	TGTGAAACOC	AGAAOCCAGT	8300
erProArgLe	uGlnLeuSer	AsnGlyAsnA	rgThrLeuTh	rLeuPheAsn	ValThrArgA	snAspThrAl	aSerTyrLys	CysGluThrG	lnAsnProVa	
					<b>Intron 3</b>					
GAGTGCAGG	CGCAGTGATT	CAGTCATOCT	GAATGTOCTC	Tgtgagtata	tctgtcctc	tctggccag	gctgccagcc	aaatocacag	ggccagaxxg	8400
lSerAlaArg	ArgSerAspS	erValIleLe	uAsnValLeu	T						
ggattttca	gtccctctca	ggttcaagta	cacagaccct	caacxtgga	catcxagact	gtctgtgact	ttctgcccag	aaaa.... (ca 0.4 kb)...		8900*
								<b>RI domain</b>		
ttagagggac	actgtggtcc	ttcatagac	caggaacttc	cacttccctc	tgacaatc	acctgtggct	ttattttggt	tgctccagAT	GGCOGGATG	9000
								yr	GlyProAspA	
COOCCOCCAT	TTCOOCTCTA	AACACATCTT	ACAGATCAGG	GGAAAATCTG	AACCTCTOCT	GCCACGCAGC	CTCTAAOCCA	CCTGCACAGT	ACTCTTGGIT	9100
laProThrIl	eSerProLeu	AsnThrSerT	yrArgSerGl	yGluAsnLeu	AsnLeuSerC	ysHisAlaAl	aSerAsnPro	ProAlaGlnT	yrSerTrpPh	
TGTCAATGG	ACTTTCOCAG	AATCCACCCA	AGAGCTCTTT	ATOCCCAACA	TCAGTGTGAA	TAATAGTGG	TCCTATACGT	GCCAGCOCCA	TAACTCAGAC	9200
eValAsnGly	ThrPheGlnG	lnSerThrGl	nGluLeuPhe	IleProAsnI	leThrValAs	nAsnSerGly	SerTyrThrC	ysGlnAlaHi	sAsnSerAsp	
					<b>Intron 4</b>					
ACTGGCOCTCA	ATAGGACOCAC	AGTCAOGAOG	ATCAGAGTCT	ATGgttaagt	gatccaogaa	gcactgacat	catgttttga	ggtggagct	gtctggtttt	9300
ThrGlyLeuA	snArgThrTh	rValThrThr	IleThrValT	yrA						
caacaagag	ccaggaagac	atcttctatc	ccagcctgtg	tccagtgggc	acaagcaaat	cccagattct	cccactgaac	ctccccaaata	tgtctctaca	9400
gactcttttc	tcttgttct	gatttctcat	ggcggggccc	agccagcctt	ggaatgtggg	goggagggctc	octcagcccc	acagccctgt	gtagtggagg	9500
aag.....	.....	.....	.....	(ca 1.6 kb).....		.....	.....	.....	.....	11,100*
aggtctctga	gcocctcagat	catgtgcat	ctgtctgtg	acgcacacac	acctgccatg	agcttttaax	gactcagttg	ggctgagagg	toggagatgc	11,200

caactctgat tgatagatgc cogtggagga atcacaggtg ccacacaggg caatcttctc tctgttatct gcacagCAGA GGCACOCMA COCTTCATCA 11,300  
laG1 uProProLys ProPheIleT

**A2 domain**

CCAGCAACAA CTCGAACCCC GTGGAGGATG AGGATGCTGT AGOCTTAAOC TG1GAAOCTG AGATTTCAGAA CACAACCTAC CTGTGGTGGG TAATAATCAG 11,400  
hrSerAsnAs nSerAsnPro ValGluAspG luAspAlaVa lAlaLeuThr CysGluProG luIleGlnAs nThrThrTyr LeuTrpTrpV alAsnAsnGl

GAGCTOCCG GTCAGTCCA GCGTGCAGCT GTCCAATGAC AACAGGAOCC TCACCTCTACT CAGTGTCCACA AGGAATGATG TAGGAOCCTA TGAGTGTGGA 11,500  
nSerLeuPro ValSerProA rgLeuGlnLe uSerAsnAsp AsnArgThrL euThrLeuLe uSerValThr ArgAsnAspV alGlyProTy rGluCysGly

**Intron 5**

ATCCAGAAOC AATTAAGTGT TGACCACAGC GACOCAGTCA TCCTGAATGT CCTCTgtgag tatcttctgt tctctgtggt ctcaggctcg gagcccaaat 11,600  
IleGlnAsnG luLeuSerVa lAspHisSer AspProValI leLeuAsnVa lLeuT

ccacatagcc aaagt..... (ca 0.5 kb)..... 12,100\*

**E2 domain**

aggggxacac tgttgccctt tcacagacca ggagcttccc ctttgcctcg atgacattca cctgtggccc tattctcttt getccagATG GOCCAGACGA 12,200  
yrG lyProAspAs

CCCCACCATT TOCCOCTCAT ACAOCTATTA COGTCCAGGG GTGAACCTCA GOCTCTOCTG CCATGCAGOC TCTAACCCAC CTGCACAGTA TTCTGTGCTG 12,300  
pProThrIle SerProSerT yrThrTyrTy rArgProGly ValAsnLeuS erLeuSerCy sHisAlaAla SerAsnProP roAlaGlnTy rSerTrpLeu

ATTGATGGGA ACATCCAGCA ACACACACAA GAGCTCTTTA TCTCCAACAT CACTGAGAAG AACAGCGGAC TCTATAOCTG CCAGGOCAT AACTCAGOCA 12,400  
IleAspGlyA snIleGlnG lnhisThrGln GluLeuPheI leSerAsnIl eThrGluLys AsnSerGlyL euTyThrTyrCy sGlnAlaAsn AsnSerAlaS

**Intron 6**

GTGGCCACAG CAGGACTACA GTCAAGACAA TCACAGTCTC TGgtaagtgg atccctggac cgttagcaat atgttctgga goggaatctg tctggttttc 12,500  
erGlyHisSe rArgThrThr VallysThrI leThrValSe rA

agaaaagagc caggaagaaa ttttctttcc tagtatgcat ccaatgggca caagcaatcc caaattcaat cctgagcact cccaattgt ctctacaac 12,600  
actctt..... (ca 1.6 kb)..... 14,200\*

aagcagcogg ccttacagtc tctgagcctc catatcatcg tacatctgtc ttgtgatata cacactgcc atgggctttt aaggactcgg gtgggctgaa 14,300

**A3 domain**

gggtgggagt tgccaactct gattgaaaga tgctgtgag gaatcaaaag tggcacacag ggaactcttc tctctgttat ctgcacagCG GAGCTGCCA 14,400  
la GluLeuProL

AGOOCTOCAT CTCAGCAAC AACTOCAAC COGTGGAGGA CAAGGATGCT GTGGCCTTCA CCTGTGAAOC TGAGGCTCAG AACACAACCT AOCCTGTGGT 14,500  
ysProSerIl eSerSerAsn AsnSerLysP roValGluAs pLysAspAla ValAlaPheT hrCysGluPr oGluAlaGln AsnThrThrT yrLeuTrpTr

GGTAAATGGT CAGAGCCTOC CAGCTCAGTC CAGGCTGCAG CTGTCCAATG GCAACAGGAC CCTCACTCTA TTCATGTICA CAAGAAATGA CCACAAGACC 14,600  
pValAsnGly GlnSerLeuP roValSerPr oArgLeuGln LeuSerAsnG lyAsnArgTh rLeuThrLeu PheAsnValT hrArgAsnAs pAlaArgAla

**Intron 7**

TATGTATGTG GAATCCAGAA CTCAGTGAAT GCAAACCGCA GTGAOCCAGT CAOCTGGAT GTOCTCTgtg agtatctctg ttctctctgt gacctggttt 14,700  
TyrValCysG lyIleGlnAs nSerValSer AlaAsnArgS erAspProVa lThrLeuAsp ValLeuT

..... (ca 1.8 kb)..... 16,500\*

atttggactt ttttaacacag xattgggaca gxattcagag gxacactgtg gcxxttctac aatcaggagc ttccoccttc ctctgatgac atcacctgtg 16,600

**E3 domain**

gctttgttct ctttgttcca gATGGGCOGG ACACOOCCAT CATTTCOOCC OCAGACTGCT CTTAOCCTTC GGGAGOGAAC CTCAOCTCT OCTGOCCTC 16,700  
yrGlyProA spThrProIl eIleSerPro ProAspSerS erTyrLeuSe rGlyAlaAsn LeuAsnLeuS erCysHisSe

GGCCTCTAAC CCATCOCCGC AGTATCTCTG GGTATCAAT GGGATACCC AGCAACACAC ACAAGTCTTC TTAATOGCAA AAATCCAGCC AAATATAAC 16,800  
rAlaSerAsn ProSerProG lnTyrSerTr pArgIleAsn GlyIleProG lnGlnHisTh rGlnValLeu PheIleAlaL ysIleThrPr oAsnAsnAsn

**Intron 8**

GGGAOCTATG CCTGTTTTGT CTTAACTTGT GCTACTGGCC GCAATAATTC CATAGTCAAG AGCATCACAG TCTCTGgtaa gtggctccct ggagcatcag 16,900  
GlyThrTyrA laCysPheVa lSerAsnLeu AlaThrGlyA rgAsnAsnSe rIleValLys SerIleThrV alSerA

**NcoI**

catcatattc tggggtggag tctatctggt tctcaccaaa gagccaagaa gacattttct ttcccagctc gtgttccatg ggcacaagga aatcccaaat 17,000  
tctatctga gxcocctcac ..... (ca 4.8 kb)..... 21,800\*

**M domain**

tccatgacgg aogattcagc AICTGGAAT TCTOCTGGT TCTCAGCTGG GGCAGTCTT GGCATCATGA TTGGAGTGTG GGTGGGGTT GCTCTGATAT 21,900  
l aSerGlyThr SerProGlyL euSerAlaG lYAlaThrVal GlyIleMetI leGlyValLe uValGlyVal AlaLeuIle\*

**Intron 9**

AGCAGOOCTG GTGTAGTTC TTCAITTCAG GAAGACTGgt aggtataatg gcctttctc ttgttctggt tctctcag.. (ca 2.0 kb)..... 24,000\*

**3'UT**

aattatcatc agatttttaa ctgtactcat tttaatctt gtcattcaca gACAGTGTG TTGCTCTTC CTTAAAGCAT TTGCAACAGC TACAGICTAA 24,100

**Alu repeat**

AAITGCTTCT TTACCAAGGA TATTTACAGA AAAGACTCTG ACCAGAGTC GAGACCATCC TAGCCACAT CGTGAACCC CATCTCTACT AAAATACAA 24,200  
AAATGAGCTG GGCTGGTGG CGGCAOCTG TAGTCCAGT TACTGCGGAG GCTGAGGAG GAGAATGCT TGAACCGGG AGGTGGAGAT TGCAGTGGC 24,300  
CCAGATGCA CCACTGCAGT CAGTCTGGCA ACAGAGCAAG ACTCCATCTC AAAAAGAAAA GAAAAGAAGA CTCGTGCTG TACTCTGAA TACAAGTTC 24,400  
TGATACCACT GCACTGTCTG AGAATTTACA AAAGTTAAT GAACTAACG ACAGTTCAT GAACTGCCA CCAAGATCAA GCAGAGAAAA TAATTAATTT 24,500  
CATGGGACTA AATGAACTAA TGAGGATAAT ATTTTCATAA TTTTITATTT GAAATTTTC TGATTTCTTA AATGCTCTG TTCCAGATT TCAGGAAACT 24,600  
TTTTTCTTTT TAAGCTATOC ACAGCTTACA GCAATTGATA AAATAACTT TTGGAACAA AAATGAGAC AITTTACATTT TCTCCCTAGT GGTCGCTCA 24,700

**Poly A**

GACTTGGGAA ACTATTCAAT AATATTATA TTGTATGSTA ATATAGTTAT TGCACAAGTT CAATAAAAAT CTGCTCTTTG TATGACAGAA TACATTTGAA 24,800

**Poly A**

AACATTTGTT ATATTACCAA GACTTTGACT AGAATGTGT AITTTAGGAT ATAAACCCAT AGGTAATAA OCCACAGGTA CTACAACAA AGTCTGAAGT 24,900

**Alu repeat**

CAGCCTTGGT TTGGCTTCC AGTGTCAAT AACTTCTAA AAGTTAATC TGAGATTCT TATAAAAACT TCACGCAAG CAACTTTTAA AAAGTCTG 25,000  
TGGGOCGGC CGGTGGCTC AGOCTCTTAA TCCAGCACT TCTTACGSC GAGG ..... (ca 180 bp Alu repeat) ..... 25,200\*

**Poly A**

AGTGAAGCAA GATACTGGC TGCACTCCAG CCTGXGAGC AAAGTGAAG TCGTCAAAA AAAAATAAAA GCTATGTTGG TCAGTCACTA CTCTTGCCG 25,300

**Poly A**

CAGTTATGAA AAGAATGAG CCAAGTTGAT GAAATAAAC TTATTTTGAA AACAAATTT TCTTATTTGG ATATATCTTTG GTAGAAATAG AGATGCTAT 25,400

**"NCA-like" exon**

GAAGAGAAAT TATGTTGAAA AAAAACTATA GTACACCTGT TATGAGACTG TCACCTTTGTA CATGTTGAG TTTTATATT CCAOCTGTAG ACTAGAGTGG 25,500

**EcoRI**

ACCATGAATT C

```

- 1 Leader                -22                1 N-terminal Domain
MESPSAPPHRWCIPWQRLLLTASLLITFWNPPTTA KLTIESTP.....

      108 A1                201 B1
EATGOFRVYPELPKPS.....VILNVLYGDAP.....

      285 A2                379 B2
TIVTTTTIVYAPEPKPF.....VILNVLYGDDDP.....

      464 A3                557 B3
TIVKTTIVSAELPKPS.....VILDVLYYGDDTPI.....

      642 M-domain          668
SIVKSIIVSASGTSPGLSAGATVGMIGVLVGVALI

```

FIG. 3. Correlation of exons with protein domains in CEA. The amino acid sequence, including the leader and membrane peptides, is shown in single-letter code over the regions of exon/intron boundaries. The amino acids whose codons are split by exon/intron junctions are shown in outline. The N-terminal leader peptide is split at codon -22, a common feature in immunoglobulin-related genes. The N terminus of the mature protein is lysine (K). Each of the immunoglobulin-like domains (N-terminal, A1, B1, A2, B2, A3, B3) ends with a predicted  $\beta$ -strand (underlined) and begins (except for the N-terminal domain) with a predicted interconnecting loop, rich in proline residues (bold). The membrane peptide (M-domain), which serves as a signal for attachment of the phosphatidylinositol glycan moiety, is at the beginning of a separate exon.

activity in both cell lines. These values ranged from 4 to 20% of the pSV2CAT activity. The CEA constructs, however, exhibited a much higher CAT expression in the SW403 cells, in which the mean value was approximately 110% for the pCEA424/2CAT construct, compared with the HeLa cells, in which the values ranged from 5 to 12% of the pSV2CAT activity for all CEA constructs. The expression levels of the longer CEA constructs (pCEA832/2CAT and pCEA3300/2CAT) were also elevated in SW403 cells compared with HeLa cells (50 and 40% of the pSV2CAT activity, respectively). The constructs for both genes in which the promoters were in the inverse orientation (pNCA48/1246CAT and pCEA2/832CAT) showed no promoter activity (<1%) in either cell line. The pBLCAT3 vector, which lacks a promoter entirely, was also not active (<1%).

## DISCUSSION

We have isolated a cosmid clone (cosCEA1) from a human genomic library; this clone contains the complete coding sequence for CEA, as determined by restriction endonuclease mapping and DNA sequencing (Fig. 1 and 2). Another group recently reported the independent isolation and characterization of an identical clone from the same genomic library (T. C. Willcocks and I. W. Craig, Abstr. XVIIth Meet. Int. Soc. Oncodevel. Biol. Med. 1989, abstr. no. 1P-1, p. 51). The exon structure is the same as that reported for other members of the CEA gene family for the immunoglobulin variable- and constant-like domains (33-35, 50, 51) and, likewise, for other members of the immunoglobulin supergene family (58). All of the immunoglobulin-like domain exon sequences reported here are present in the mature CEA mRNA, whose sequence has been determined elsewhere (2, 4, 36, 59). Hybridization studies with restriction endonuclease fragments from the N-terminal and repeat domains of a CEA cDNA do not indicate the existence of additional immunoglobulin variable- or constant-like domain exons in the CEA gene.

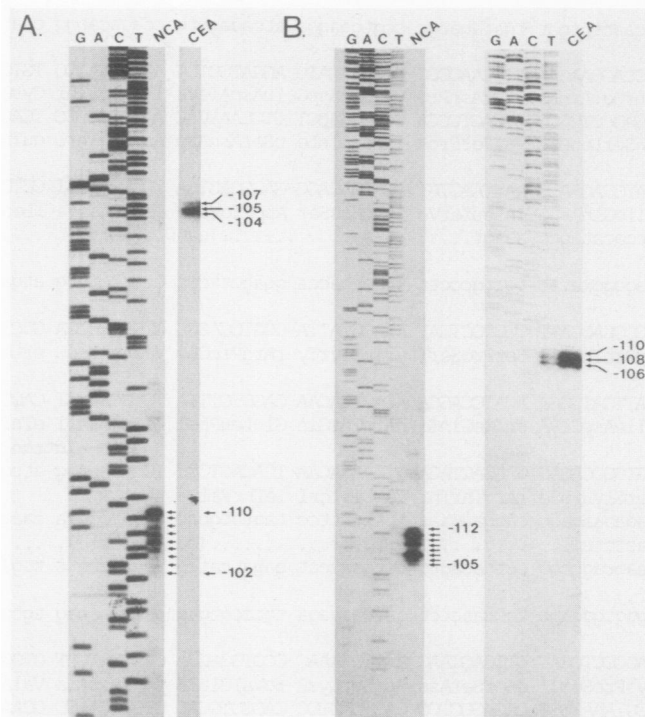


FIG. 4. Determination of the transcriptional starts of the NCA and CEA genes through primer extension (A) and S1 nuclease (B) analyses. As length markers, DNA sequences of the corresponding genomic regions, with the same NCA (A) or NCA and CEA (B) oligonucleotide primers for each gene as for the transcriptional start analyses (NCA5' and CEAL, respectively), were used to allow the start positions to be determined exactly. In both cases, the numbers indicate the transcriptional start sites with respect to the initiation codons of each gene.

Analysis of the 3' end of the gene has revealed that the hydrophobic membrane domain is encoded by a separate exon (M/3'), which also contains the first 39 nucleotides of the 3' untranslated region (Fig. 1 and 2). This region is homologous to a corresponding section of the 3' untranslated region found in NCA cDNA sequences (2, 31, 47). After this, the sequences of the 3' untranslated regions of the CEA and NCA mRNAs are no longer homologous. The point of their divergence correlates exactly with the end of this M/3' exon in the CEA gene (Fig. 2). The following exon in the CEA gene contains the rest of the CEA 3' untranslated region (Fig. 1 and 2, 3' exon). Preliminary characterization of the region directly downstream from this exon has revealed a sequence which shows homology to the NCA 3' untranslated region ("NCA" 3' exon), including a conserved *EcoRI* restriction endonuclease site (Fig. 2; data not shown). A consensus intron acceptor site (30) exists at the 5' end of this NCA-like exon (Fig. 2), which correlates with a corresponding splice site in a related PSG gene (33). This PSG gene also contains both CEA and NCA-like 3' untranslated regions, thus confirming the model, originally proposed by Oikawa et al. (33) and extended by Zimmermann et al. (61), that all genes belonging to the CEA family contain such a complex 3' unit. Therefore, variability in the 3' untranslated regions in the CEA and NCA mRNAs is probably due to differential splicing of their corresponding genes. These results also indicate that a CEA transcript which contains the NCA-like 3' untranslated region could exist and that the probe from the



FIG. 5. Comparison of the nucleotide sequences from the putative promoter regions of the NCA, CEA, and CGM1 genes. The numbers indicate the distance in nucleotides from the initiation codon for each gene. Gaps have been introduced to allow optimal alignment. Identical nucleotides are indicated by dots. The cluster of transcriptional start sites determined for CEA and NCA in the S1 nuclease assays (see Fig. 4 and text) are designated (arrows). Consensus TATA sequences for the NCA and CEA genes, as well as the initiation codons, are shaded grey.

3' untranslated region of an NCA cDNA clone (60) may not be NCA mRNA specific.

Recently, we found that after stable transfection of this CEA cosmid clone into Chinese hamster ovary (CHO) cells, a 180,000-molecular-weight glycosylated CEA molecule was expressed on the cell surface (L. J. F. Hefta, H. Schrewe, J. A. Thompson, S. Oikawa, H. Nakazato, and J. E. Shively, Cancer Res., in press). These results show that a functional promoter is probably present in the 3,300-nucleotide region upstream of the translational start contained within this cosmid clone (Fig. 1). Although a rapid sequence divergence between the human and rodent CEA families has been reported, which indicates a parallel but independent evolution of the genes in different mammalian orders (39), these expression results suggest that the *trans*-acting factors and *cis*-acting elements responsible for activating the CEA gene are conserved enough between humans and rodents to allow expression across species. Therefore, closer analysis of the regulation of human CEA gene expression in a rodent system is feasible. Furthermore, the encoded CEA molecule was apparently posttranslationally modified through addition of a phosphatidylinositol glycan tail, as for the native CEA molecule (Hefta et al., in press). These results indicate that all the information needed for the correct expression of the CEA gene is contained within cosCEA1.

To define the actual portion of the 5' untranslated region which is required for the promoter activity of the CEA gene and, for comparison, that of a closely related NCA gene, we carried out functional tests by placing restriction endonuclease fragments of various lengths from the putative promoter

regions of both genes upstream of the CAT reporter gene and assaying for CAT activity in a transient transfection assay in two different human cell lines (Fig. 6). For this purpose, we chose the CEA-producing adenocarcinoma cell line SW403 and, as a negative control, the HeLa cell line. No significant differences in the promoter activities could be determined between the cell lines with the NCA constructs used. These seem to contain only a basal level of promoter activity, especially for the shortest construct, which showed minimal activity (Fig. 6). On the other hand, the CEA promoter constructs showed an enhanced expression of the CAT gene in SW403 cells, which was nine times greater than in HeLa cells, when the shortest construct was used (Fig. 6, pCEA424/2CAT). It appears that *cis* regulatory sequences, which are responsible for this enhancement, along with a functional transcription initiator, are both present within the first 424 nucleotides upstream of the translational start. When the sequences in this region of the CEA and NCA genes were compared, homology was found (Fig. 5 and 6); however, regions showing stronger sequence divergence also exist in the region upstream of -240 nucleotides from the translational start of the CEA gene, which may be of interest regarding the differential regulation of expression of the two genes. Further experiments must be designed to analyze these regions more closely. It is also interesting that the longer CEA constructs are approximately 50% less active in their promoter activities in SW403 cells and 25% less active in HeLa cells than is the pCEA424/2CAT construct (Fig. 6). A possible explanation for this phenomenon is that a silencer region could exist between nucleotides



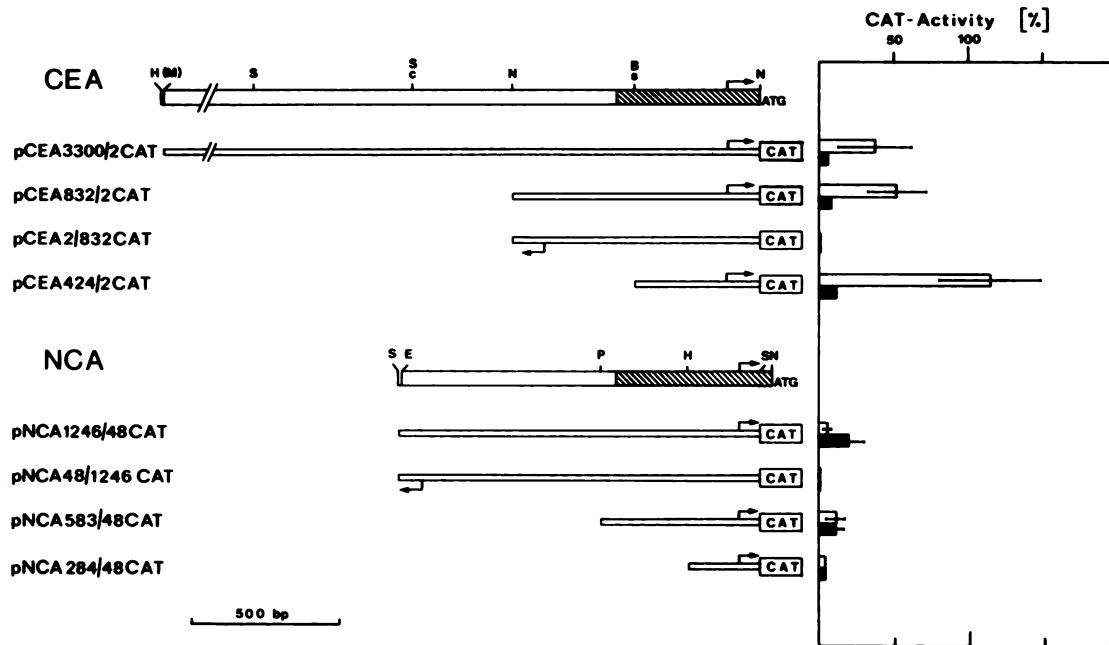


FIG. 6. Determination of the CEA and NCA gene promoters through transient-transfection assays in SW403 and HeLa cells. The regions analyzed for both genes are indicated ( $\square$ ), and the positions of the initiation codons are shown. Regions of sequence homology are shown ( $\text{▨}$ ). The following restriction endonuclease sites were used for the constructs: *Bst*XI, (Bs), *Eco*RI (E), *Hind*III (H), *Nco*I (N), *Pst*I (P), *Sst*I (S), and degenerate *Msp*I cloning sites [(M)]. A rare-cutting restriction endonuclease site (*Sac*II) found in the C+G-rich CEA promoter is shown (Sc). The arrows indicate the transcriptional starts and orientation. The fragments of each gene promoter spliced in front of the promoterless CAT reporter gene are shown ( $\square$ ). The CAT activity was determined for each construct after transient transfection into SW403 cells ( $\square$ ) and HeLa cells ( $\blacksquare$ ). The activities are expressed as a percentage of the CAT activity obtained with the plasmid pSV2CAT (see text), which was equivalent to a mean of 30,000 dpm in SW403 or 50,000 dpm in HeLa cells (ca. 30 and 50% conversion of the acetyl coenzyme A added, respectively; this was the limiting factor). Up to five separate assays were carried out for each construct to confirm reproducibility. Standard deviations are indicated with bars.

–424 and –832 upstream from the translational start, which reduces the activities in both cell lines through interaction with common *trans*-acting regulatory factors. Such silencer sequences have indeed been described for other genes (8, 9). However, further studies must be carried out to analyze this in more detail.

As found here for CEA and NCA, a number of other eucaryotic genes have also been reported which do not contain obvious TATA boxes. The promoters of such genes can be divided into two classes (42). The members of the first class are G+C rich and are found primarily in housekeeping genes (41). These promoters usually contain several transcription initiation sites spread over a fairly large region, as well as potential binding sites for Sp1 (11). The members of the second class are not G+C rich, are not constitutively active, but are regulated during differentiation or development and initiate transcription at only one or a few tightly clustered start sites (42). Included in this class are a number of genes that are regulated during mammalian immunodifferentiation, e.g., the T-cell receptor  $\beta$ -chain genes (1) and the  $V_{preB}$  gene (22), as well as some *Drosophila* homeotic genes (6, 38, 43). The CEA and NCA genes show a closer resemblance to this latter group, because their promoters are not obviously G+C rich, they contain no identifiable Sp1-binding sites, they reveal only a few tightly clustered start sites, and, most importantly, they are not constitutively expressed.

Future experiments will concentrate on more closely defining the various regions of interest in the promoters and, in parallel, will be directed toward identifying regions which

possibly convey an oncofetally regulated expression pattern of the CEA gene.

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