[CANCER RESEARCH 49, 2017-2021, April 15, 1989]

A Mouse Analogue of the Human Carcinoembryonic Antigen[1]

N. Beauchemin,[2] C. Turbide, D. Afar, J. Bell, M. Raymond, C. P. Stanners, and A. Fuks

McGill Cancer Centre [N. B., C. T., C. P. S., A. F.] and Department of Biochemistry [D. A., J. B., M. R., C. P. S.], McGill University, Montreal H3G 1Y6, Canada

ABSTRACT

Functional human carcinoembryonic antigen (CEA)-like genes have been shown to be present in the mouse. Southern analyses of murine DNA using both human and murine CEA complementary DNA probes have revealed the presence of multiple CEA-like genes, while analyses of RNA from different mouse tissues showed CEA-like transcripts in adult colon and liver. Furthermore, a CEA-like protein, immunoprecipitable with a rabbit polyclonal serum raised against human CEA, has been detected in adult murine colon tissue. Several murine CEA complementary DNA clones have been isolated from a murine colon complementary DNA library, and characterization of one such clone demonstrates that both the *N*-terminal and the internal domains have been conserved between the two species. The existence of a murine counterpart of CEA strengthens the case for an essential function for this human tumor marker and provides an experimentally amenable system for elucidation of its biological properties.

INTRODUCTION

CEA[3] is a large cell surface glycoprotein (M_r 180,000) first described in 1965 by Gold and Freedman as an antigen expressed in tumors of the human gastrointestinal tract (1) and in the fetal digestive system (2). This or related antigens have since been detected in a wide range of human malignancies including breast, lung, and ovarian cancers (3, 4). Assays that measure the serum or plasma levels of CEA have been useful in assessing the prognosis and monitoring the progress of cancer patients (5). CEA is also a member of a large and complex gene family (6). NCA, for example, is a family member specifically found in breast tumors, chronic myelogeneous leukemia, and normal gall bladder (7, 8) as well as in normal spleen and lung (9, 10).

The cDNAs corresponding to CEA and NCA mRNAs have recently been cloned, and their nucleotide sequences and predicted translation products have been determined (11–15). Both proteins belong to the immunoglobulin supergene family, and initial studies point to an intercellular adhesion function for CEA.[4]

In depth investigations of the function of CEA and its role in malignancy will require animal models, preferably murine, allowing approaches such as transgenesis, controlled carcinogenesis, and *in situ* assessment of expression during development. Therefore, we have undertaken a search for a mouse counterpart of CEA. Previous studies of animal models have identified putative counterparts of CEA or NCA using immune sera or specific monoclonal antibodies. A putative rat CEA was detected in chemically induced colonic adenocarcinomas, embryonic tissues, and, in low concentrations, normal adult tissues (16). An antigen which is immunologically closely related to human NCA and which cross-reacts with CEA has also been identified in lung and spleen tissues of Macaca monkeys (17), and blood samples from other primates including gorilla, orangutang, black ape, mangabey ape, baboon, and chimpanzee exhibited CEA-related substances (18). We report here the identification and initial characterization of a mouse counterpart of CEA present in normal adult colon and in liver tissues.

MATERIALS AND METHODS

Cell Culture. Cells of the human colonic adenocarcinoma line (LS-180) (19) and a line of L-strain mouse fibroblasts (LTA) were grown at 37°C in monolayer in α-minimal essential medium (20) supplemented with 10% fetal bovine serum and asparagine · H_2O at 50 μg/ml.

^{32}P-labeled Probes. Hybridization probes for Northern and Southern analyses consisted of either an 800-base pair *Nco*I-*Bam*HI restriction fragment comprising the *N*-terminus and most of the first internal repeating domain of the human CEA cDNA (11), a 1.43-kilobase *Eco*RI restriction fragment specific for the 3′-untranslated region of the human NCA cDNA (7), or a 1.45-kilobase *Eco*RI restriction fragment of a murine CEA cDNA clone. These fragments were extracted from 0.8% low-melting agarose gels and ^{32}P-labeled using the random primer technique of Feinberg *et al.* (21).

DNA Preparation and Southern Analyses. Genomic DNA was prepared (22) from two mouse leukemic lines L1210SS and L1210DN (23), a mouse fibroblast line LTA 42.2 (24), and normal human liver tissues. DNA was digested with *Eco*RI restriction endonuclease, and the digestion products were electrophoresed on a 1% agarose gel. The gels were transferred to a hybridization membrane (Zeta-probe, Bio-Rad; or Hybond-N, Amersham). Identical mouse Southern filters were hybridized with the ^{32}P-labeled CEA probe in 5× SSPE, 1× Denhardt's solution (22), 100 μg/ml of heat-denatured salmon testis DNA, and either 30%, 40%, or 50% formamide at 40°C for 48 h. The human genomic filters were hybridized at 42°C for 18 h under the same conditions but with 0.5% SDS, 10% dextran sulfate, and 30% formamide. The murine fibroblast genomic DNA was hybridized using the above conditions with 50% formamide at 42°C. Filters were exposed to Kodak XRP X-ray films for 18 to 36 h after washing.

RNA Preparation and Northern Analyses. Fresh tissues were collected from either pregnant or nonpregnant female CD-1 mice and immediately frozen in liquid nitrogen. Approximately 2 g of tissue were powdered using a mortar and pestle kept at −40°C, and the fine powder was vortexed into a solution of 4.0 M guanidium isothiocyanate containing 25 mM sodium citrate (pH 7.0), 0.5% Sarkosyl, and 0.1 M β-mercaptoethanol (25). Poly A$^+$ RNA was purified on oligo dT columns (26). Total or poly A$^+$ RNA was electrophoresed on 1.5% agarose gels containing 1.1 M formaldehyde, 10 mM sodium phosphate buffer (pH 7.4), and 1 mM EDTA; stained with acridine orange; and transferred to either nitrocellulose or Hybond-N (Amersham Corp.) membranes. Hybridization was done for 18 h at 37°C or 42°C in 5× SSPE, 1× Denhardt's solution, 30% or 50% formamide, 150 μg/ml of heat-denatured salmon testis DNA, 2 mM sodium pyrophosphate, 0.1% SDS, 10% dextran sulfate for the nitrocellulose membranes, or 5% dextran sulfate for the Hybond-N membranes using 2.5×10^6 to 4.0×10^6 cpm/ml of the ^{32}P-labeled probes. The filters were washed up to a final stringency of 0.1× SSC and 0.1% SDS at 50°C. 18S rRNA (2.0 kilobases) and 28S rRNA (4.5 kilobases) were used as markers.

cDNA Library and Isolation of Murine CEA cDNA Clones. cDNA was synthesized, as described previously (10), from 5 μg of poly A$^+$ RNA isolated from murine adult colon, ligated to dephosphorylated *Eco*RI-digested λgt10 DNA, and packaged with Gigapack Plus extracts (Stratagene) to yield infectious virus. λgt10 recombinant bacteriophages

Received 3/29/88; revised 9/16/88; accepted 1/4/89.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants to C. P. S., J. B., and A. F. from the Medical Research Council of Canada and the National Cancer Institute of Canada.

[2] Recipient of a fellowship from Fonds de la Recherche en Santé du Québec.

[3] The abbreviations used are: CEA, carcinoembryonic antigen; NCA, normal cross-reacting antigen; cDNA, complementary DNA; SSPE, 0.18 M NaCl:1 mM EDTA:10 mM sodium phosphate (pH 7.7); SDS, sodium dodecyl sulfate; poly A$^+$ RNA, polyadenylate-containing RNA; SSC, standard saline citrate.

[4] S. Benchimol *et al.*, manuscript in preparation.

were plated on *Escherichia coli* C600 hflA 150 (27). Five × 10^5 clones were screened by duplicate plaque hybridization using a human *Nco*I-*Bam*HI ^{32}P-labeled restriction fragment of CEA cDNA (11) under low stringency conditions (30% formamide, 37°C) as described above. Filters were washed up to a final stringency of 0.1 SSC and 0.1% SDS at 50°C and exposed to Kodak XRP X-ray films. Positive cDNA clones were purified by three successive replatings on *E. coli* C600 hflA 150.

DNA Sequence Determination. Murine CEA cDNA inserts were subcloned into unique sites of the pGEM4 plasmid (Promega; Biotec). Two μg of plasmid DNA were denatured by a 5-min incubation in 0.2 N NaOH and 2 mM EDTA, followed by neutralization and ethanol precipitation. The DNA was then hybridized with 30 ng of either the SP6 or T7 promoter primer (Promega) and sequenced by the dideoxy chain termination method (28) using Sequenase (United States Biochemicals). Compression regions were verified by resequencing with dITP and confirmed by opposite strand sequence.

Sequence Analysis. Nucleotide and amino acid sequences were analyzed using the DNAsis and Prosis programs (LKB) or alternatively the Deverreux (29) programs.

Western Blotting of Mouse Proteins. Mice were sacrificed, and the specified organs were rapidly removed, incubated in ice-cold hypotonic buffer [20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.2):3 mM $MgCl_2$:3 mM KCl:aprotinin (2 μg/ml):phenylmethylsulfonyl fluoride (200 μg/ml)] for 20 min on ice, and then disrupted with 15 strokes at 700 rpm using a Heidolph tissue homogenizer. The homogenate was then subjected to centrifugation (1,500 × *g* for 5 min), and the subsequent supernatant was subjected to centrifugation at 75,000 × *g* for 30 min to produce a membrane-enriched pellet. The pellet was resuspended in hypotonic buffer to determine the protein concentration of this fraction, and appropriate amounts of protein were precipitated with icecold 10% trichloroacetic acid. The precipitate was washed with −20°C acetone, air dried, dissolved directly into SDS-sample buffer, and heated at 100°C for 1 min. Proteins were resolved on 7.5% SDS-polyacrylamide gels and transferred to nitrocellulose membranes as described previously (30). Immunoreactive polypeptides were identified on the nitrocellulose blots using a rabbit polyclonal antiserum raised against purified human CEA (31). This antibody has not been absorbed with human tissue samples and may thus cross-react with NCA and other members of this protein family. Antigen-antibody complexes were visualized using an alkaline phosphatase-conjugated goat antibody directed against rabbit IgG.

RESULTS

Murine Analogues of Human CEA Genes. In order to search for a murine analogue of the human CEA gene family, we probed mouse *Eco*RI-digested genomic DNA filters with the CEA cDNA restriction fragment corresponding to the *N*-terminal portion and most of the first internal repeating domain. The autoradiogram yielded as many as 14 bands when hybridized at low stringency (30% formamide, 40°C; Fig. 1*a, Panel A*), while 2 bands remained at high stringency (40% and 50% formamide, 40°C; Fig. 1*a, Panels B* and *C*). Correspondingly, human *Eco*RI-digested genomic DNA preparations contained 14 to 17 bands when probed under the same low stringency conditions (Fig. 1*c*). The band patterns observed were different in the mouse and the human DNA preparations. Probing of murine *Eco*RI-digested genomic DNA under stringent conditions (50% formamide, 42°C) using a partial murine CEA cDNA insert yielded 2 strongly hybridizing bands and 6 fainter bands (Fig. 1*b*) which points to the existence of a CEA-like gene family in the mouse as shown previously in the human.

Murine CEA Transcripts. Northern filters were prepared with poly A^+ RNA from colons of normal pregnant female mice and from a fibroblast (LTA) cell line. As can be seen in Fig. 2*A*, hybridization at low stringency with the human CEA cDNA fragment described above revealed a strong unique 3.8-kilobase band present in the colon of adult mice, but absent in the fibroblasts. By comparison, 3 bands were present in the LS-180 human colon carcinoma cell line sample (7, 11). Hybridization of the same filter with a human NCA-specific probe detected the 2.6-kilobase NCA band in the human sample, but no signal in the murine samples (results not shown).

As human CEA, NCA and other members of this gene family have been detected in various tissues (2, 6–10), we prepared total RNA from ascending, transverse, and descending colons of nonpregnant female inbred mice as well as from spleen, lung, brain, kidney, liver, and muscle tissues of these animals. Northern filters of these total RNA preparations were hybridized with the human CEA-specific probe at low stringency and with the murine CEA-specific probe under stringent conditions. A strong band was detected in the descending colon samples and a fainter signal in the ascending and transverse colon sample as well as in the liver samples (Fig. 2, *B* and *C*). Shorter transcripts were also apparent in colon samples when the murine cDNA probe was used (Fig. 2*C*). These transcripts probably correspond to other members of the murine CEA gene family, since a number of murine cDNA clones specific for

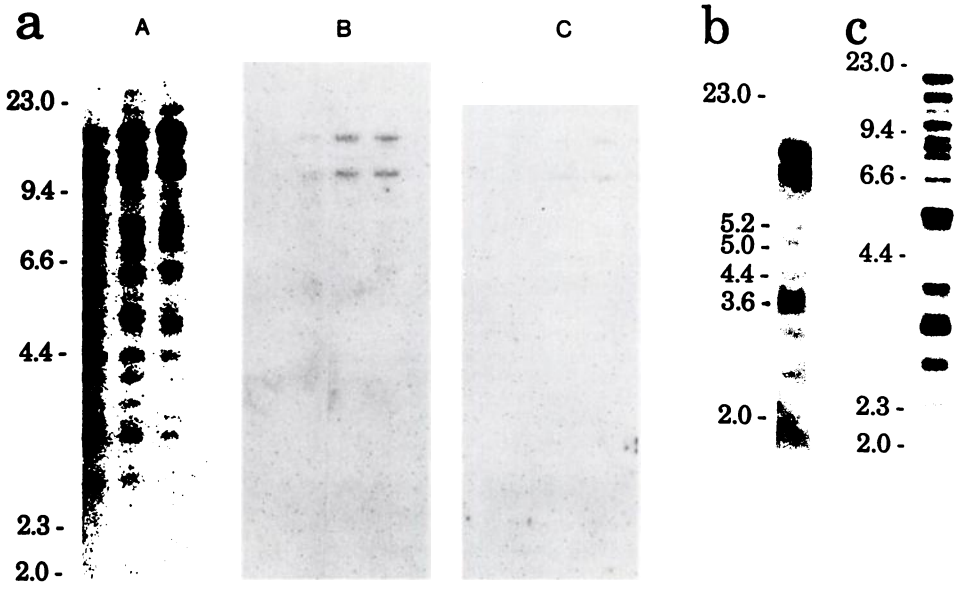

Fig. 1. Southern analysis. In *a*, 7.5 μg of each mouse *Eco*RI-digested genomic DNA preparation were resolved on an agarose gel, transferred to a Zeta-Probe membrane, and hybridized with a human CEA-specific probe as described in "Materials and Methods." Filters were washed to a final stringency of 2× SSC and 0.1% SDS at 55°C. *Lane 1*, L1210SS DNA; *Lane 2*, L1210DN DNA; *Lane 3*, LTA 42.2 DNA. *A*, 30% formamide; *B*, 40% formamide; *C*, 50% formamide. In *b*, 10 μg of mouse *Eco*RI-digested genomic DNA were separated on an agarose gel, transferred to a Hybond-N membrane, and hybridized at 42°C with a murine CEA-specific probe using the conditions described in "Materials and Methods" in a solution containing 50% formamide. Filters were washed to a final stringency of 0.1× SSC and 0.1% SDS at 50°C. In *c*, 8.5 μg of human liver *Eco*RI-digested genomic DNA were resolved on an agarose gel, transferred to a Hybond-N membrane, and hybridized at 42°C with a human CEA-specific probe using the conditions described in "Materials and Methods," in a solution containing 30% formamide. Filters were washed to a final stringency of 2× SSC and 0.1% SDS at 55°C.

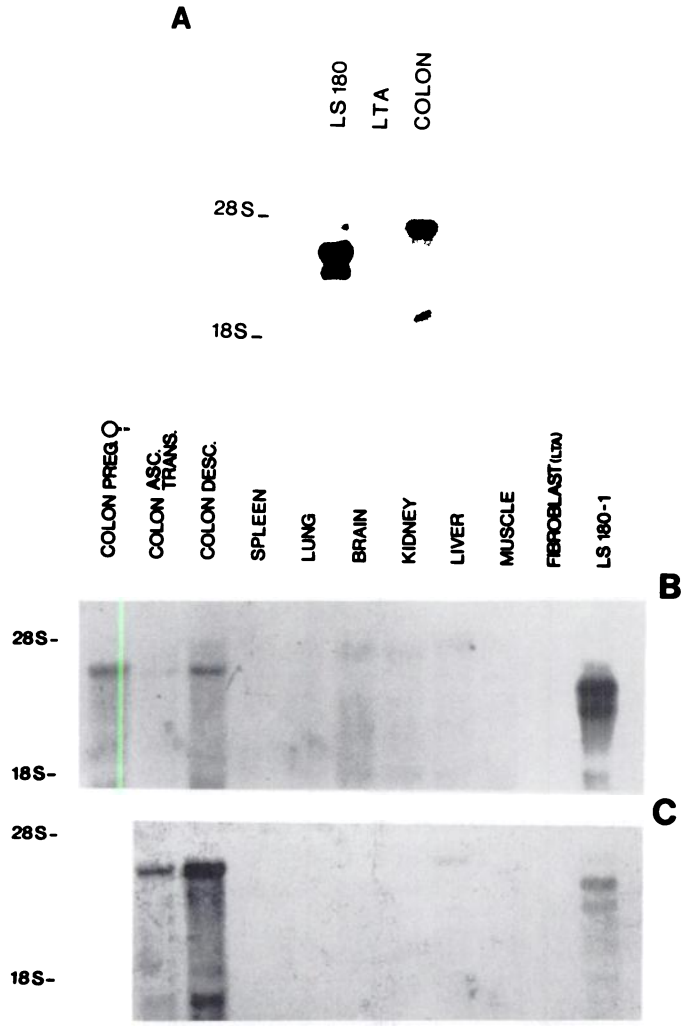

Fig. 2. Northern analysis. In *A*, samples of 5 μg of poly A⁺ RNA were separated on a formaldehyde-agarose gel, transferred to a nitrocellulose membrane, hybridized with a human CEA-specific radioactive restriction fragment, and washed using the conditions described in "Materials and Methods." RNA was extracted and purified from: *LS-180*, human colonic adenocarcinoma cell line; *LTA*, mouse fibroblast cell line; *COLON*, descending colon from pregnant CD-1 mice. In *B*, samples of 15 μg of total RNA were separated as above, transferred to a Hybond-N membrane, hybridized with a radioactive human CEA-specific restriction fragment at 37°C in a 30% formamide solution, and washed as described in "Materials and Methods." RNA was extracted from: *Lane 1*, descending colon of pregnant mice; *Lane 2*, ascending and transverse colon; *Lane 3*, descending colon; *Lane 4*, spleen; *Lane 5*, lung; *Lane 6*, brain; *Lane 7*, kidney; *Lane 8*, liver; *Lane 9*, hind-leg muscle of female mice; *Lane 10*, mouse fibroblast LTA cell line; *Lane 11*, human colonic adenocarcinoma LS-180 cell line. In *C*, samples of 15 μg of total RNA were separated, transferred as above, and hybridized with a radioactive murine CEA-specific restriction fragment at 42°C in a 50% formamide solution. Filters were washed to a final stringency of 0.1× SSC and 0.1% SDS at 50°C.

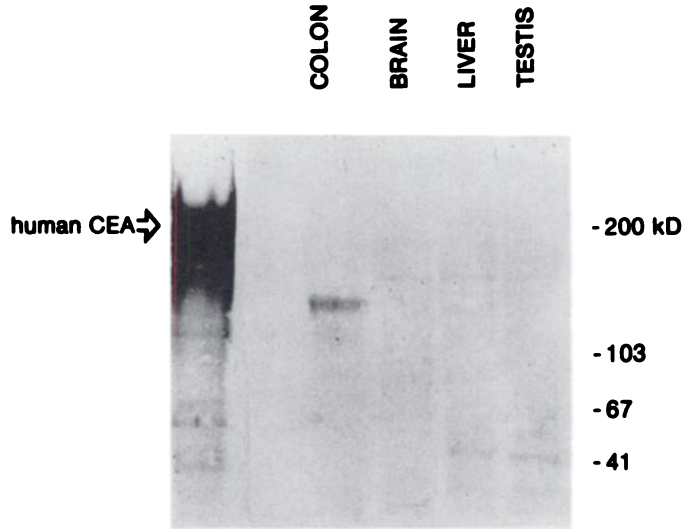

Fig. 3. Immunoblotting of mouse tissues with rabbit anti-human CEA antibodies. The indicated tissues were harvested and processed as described in "Materials and Methods." The *arrow* indicates the position of human colonic CEA protein on the gel.

shorter transcripts have been isolated from the colonic cDNA library (data not shown).

Murine CEA-like Protein. An immunoreactive M_r ~120,000 protein was detected by Western blotting in membrane-enriched preparations of adult mouse colon but not liver, testis, or brain using rabbit anti-human CEA polyclonal antibody (Fig. 3).

Murine CEA Analogue. In light of the positive results obtained in Northern analysis of murine RNA using a human CEA-specific probe, a mouse colon cDNA library was constructed and screened at reduced hybridization stringency with the human CEA cDNA fragments. Ninety-two positive clones were isolated from the 5×10^5 cDNA clones screened. The restriction map, primary sequence, and deduced translated protein product of a 1.45-kilobase incomplete cDNA clone are presented in Fig. 4 and reveal striking homology to human CEA. The partial *N*-terminal domain contains two *N*-linked glycosylation consensus sequences, one of which is perfectly aligned with a similar site in the human protein. The carboxy region of this domain has diverged relative to human CEA, although the overall conservation is highly significant (72.6% identical and conserved residues in comparison to the human *N*-terminal domain). The internal domain shows less divergence (79.4% homology to the human first internal repeating unit); the four cysteine residues thought to be involved in disulfide bridges in the human protein are conserved in the murine analogue and are found within long stretches of identical residues. A less conserved region lies between the first and second cysteines where two gaps were introduced in the human protein sequence to maximize alignment. As in the human protein, eleven potential *N*-linked glycosylation sites can be identified, although they are not always present in the same positions as in the human protein.

DISCUSSION

The results presented above demonstrate the existence of CEA-like genes in the murine system. Hybridization of genomic DNA Southern filters has revealed the presence of multiple fragments, while analysis of transcripts from different tissues of the mouse showed an abundant 3.8-kilobase transcript present in the adult colon and less abundant shorter transcripts in colon and liver tissues.

Furthermore, a murine CEA-like protein with a molecular weight of approximately 120,000 has been identified using rabbit anti-human CEA antibodies. This finding indicates that some antigenic determinants have been conserved between the human and murine CEA proteins. There are at least seven proteins to date assigned to the human CEA family by their immunological cross-reactivity and amino acid sequences (6). Our results imply that a CEA-like family may also be present in the murine system.

Analyses using differential hybridizations, restriction enzyme mapping, and DNA sequencing of various clones isolated from a mouse colon cDNA library have so far resulted in identification of four different members of the murine CEA family. These cDNAs are currently being fully characterized. Sequence

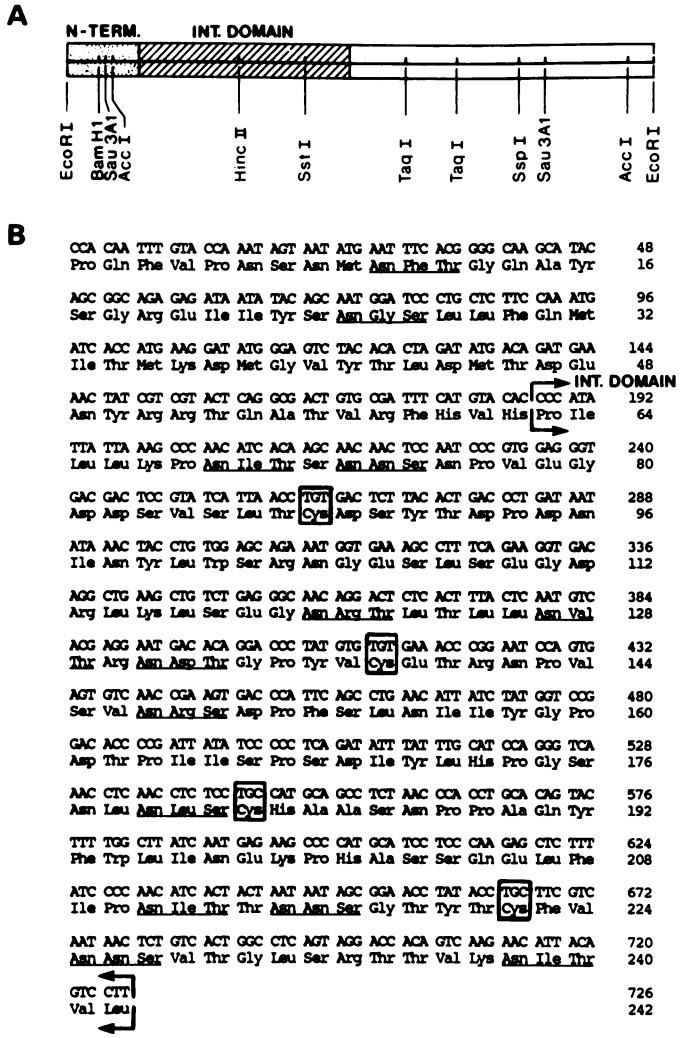

Fig. 4. Structure of a partial murine CEA cDNA (mCEA1). *A*, restriction map of a 1.45-kilobase murine CEA cDNA clone (mCEA1). The *dotted box* contains the *N*-terminal (*N-TERM.*) domain, while the *hatched box* contains the internal domain (*INT. DOMAIN*). The *open box* represents a region of open reading frame. *B*, nucleotide sequence and predicted amino acid sequence of mCEA1 cDNA. The nucleotide sequence is listed 5′ to 3′, *left* to *right*. Nucleotide and amino acid positions are shown in *side numberings*. *Underlined residues* are potential *N*-linked glycosylation sites. *Boxed areas* represent the Cys residues. The internal domain (*INT. DOMAIN*) is delineated by *arrows*. *C*, amino acid alignments of human and murine CEA predicted translation products. The human (*hCEA*) and murine (*mCEA1*) proteins have been aligned according to the BESTFIT program. :, identical amino acid; ., conserved amino acid (32); —, gaps introduced in the human sequence to maximize alignment.

data of one such clone (Fig. 4) indicate a significant conservation of amino acid residues, cysteine residues thought to form disulfide bonds, and potential *N*-linked glycosylation sites in the *N*-terminal domain as well as in the internal domain. These results validate our original hypothesis that these domains are functionally dominant and have been preserved throughout evolution.

In conclusion, our results indicate that an analogue of human CEA exists in the murine system at the genomic, transcript, and protein levels. We are currently characterizing cDNA clones of murine CEA and will use them to investigate the possible involvement of CEA in embryogenesis and development.

ACKNOWLEDGMENTS

The authors would like to thank Philippe Gros, Francois Gilbert, Danny Boucher, and Martina Timm for their technical assistance.

REFERENCES

1. Gold, P., and Freedman, S. O. Demonstration of tumor specific antigens in human colonic carcinoma by immunologic tolerance absorption techniques. J. Exp. Med., *121:* 439–462, 1965.
2. Gold, P., and Freedman, S. O. Specific carcinoembryonic antigens of the human digestive system. J. Exp. Med., *122:* 467–481, 1965.
3. Terry, W. D., Henkart, P. A., Colligan, J. E., and Todd, C. W. Carcinoembryonic antigen: characterization and clinical applications. Transplant. Rev., *20:* 100–129, 1974.
4. Shuster, J. D., Thompson, M. P., Fuks, A., and Gold, P. Immunologic approaches to diagnosis of malignancies. Prog. Exp. Tumor Res., *25:* 89–139, 1980.
5. Go, V. L. W., and Zamchek, N. The role of tumor markers in the management of colorectal cancer. Cancer (Phila.), *30* (suppl.): 2618–2623, 1982.
6. Shively, J. E., and Beatty, J. D. CEA-related antigens: molecular biology and clinical significance. Crit. Rev. Oncol. Hematol., *2:* 355–399, 1985.
7. Cournoyer, D., Beauchemin, N., Boucher, D., Benchimol, S., Fuks, A., and Stanners, C. P. Analysis of transcription of genes of the CEA family in human tissues. Cancer Res., *48:* 3153–3157, 1988.
8. Zimmermann, W., Weber, B., Ortlieb, B., Rudert, F., Schempp, W., Fiebig, H. H., Shively, J. E., von Kleist, S., and Thompson, J. A. Chromosomal localization of the carcinoembryonic antigen gene family and differential expression in various tumors. Cancer Res., *48:* 2550–2554, 1988.
9. von Kleist, S., Chavanel, G., and Burtin, P. Identification of an antigen from normal human tissue that cross-reacts with the carcinoembryonic antigen. Proc. Natl. Acad. Sci. USA, *69:* 2492–2494, 1972.
10. Mach, J. P., and Pusztaszeri, G. Demonstration of a partial identity between CEA and normal glycoprotein. Immunochemistry, *9:* 1031–1034, 1972.
11. Beauchemin, N., Benchimol, S., Cournoyer, D., Fuks, A., and Stanners, C. P. Isolation and characterization of full-length functional cDNA clones for human carcinoembryonic antigen. Mol. Cell. Biol., *7:* 3221–3230, 1987.
12. Oikawa, S., Nakazato, H., and Kosaki, G. Primary structure of human carcinoembryonic antigen (CEA) deduced from cDNA sequence. Biochem. Biophys. Res. Commun., *142:* 511–518, 1987.
13. Zimmermann, W., Ortlieb, B., Friedrich, R., and von Kleist, S. Isolation and characterization of cDNA clones encoding the human carcinoembryonic antigen reveal a highly conserved repeating structure. Proc. Natl. Acad. Sci. USA, *84:* 2960–2964, 1987.
14. Tawaragi, Y., Oikawa, S., Matsuoka, Y., Kosaki, G., and Nakazato, H. Primary structure of nonspecific cross-reacting antigen (NCA), a member of carcinoembryonic antigen (CEA) gene family, deduced from cDNA sequence. Biochem. Biophys. Res. Commun., *150:* 89–96, 1988.
15. Neumaier, M., Zimmermann, W., Shively, L., Hinoda, Y., Riggs, A. D., and Shively, J. E. Characterization of a cDNA clone for the nonspecific cross-reacting antigen (NCA) and a comparison of NCA and carcinoembryonic antigen. J. Biol. Chem., *263:* 3202–3207, 1988.
16. Abeyounis, C. J., and Milgrom, F. A thermostable antigen characteristic for carcinogen-induced rat intestinal tumours. J. Immunol. *116:* 30–34, 1987.
17. Engvall, E., Vuento, M., and Ruoslahti, E. A monkey antigen cross-reacting with carcinoembryonic antigen, CEA. Br. J. Cancer, *34:* 341–345, 1976.
18. Haagensen, D. E., Jr., Metzgar, R. S., Swenson, B., Dilley, W. G., Cox, C. E., Davis, S., Murdoch, J., Zamchek, N., and Wells, S. A. Carcinoembryonic antigen in nonhuman primates. J. Natl. Cancer Inst., *69:* 1073–1076, 1982.
19. Rutzky, L. P., Tom, B. H., and Kahan, B. D. Biological and antigenic analysis of human colon cancer cell clones. Prog. Cancer Res. Ther., *29:* 135–145, 1984.
20. Stanners, C. P., Eliceiri, G. L., and Green, H. Two types of ribosomes in mouse-hamster hybrid cells. Nature (Lond.), *230:* 52–54, 1971.
21. Feinberg, A. P., and Vogelstein, B. A technique for radiolabeling DNA

restriction endonuclease fragments to high specific activity. Anal. Biochem., *132:* 6–13 1983.
22. Maniatis, T., Fritsch, E. F., and Sambrook, J. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1982.
23. Moore, G E., Sandberg, A. A., and Ulrich, K. Suspension cell culture and *in vivo* and *in vitro* chromosome constitution of mouse leukemia L1210. J. Natl. Cancer Inst., *36:* 405–421, 1966.
24. Robertson, S. M., Ling, V., and Stanners, C. P. Co-amplification of double minute chromosomes, multiple drug resistance, and cell-surface P-glycoprotein in DNA-mediated transformants of mouse cells. Mol. Cell. Biol., *4:* 500–506, 1984
25. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. Isolation of biologically active ribonucleic acid from sources enriched in ribonucleases. Biochemistry, *18:* 5294–5299, 1979.
26. Aviv, J., and Leder, P. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA, *69:* 1408–1412, 1972.
27. Hoyt, M. A., Knight, D. M., Das, A., Miller, H. I., and Echols, H. Control of phage development by stability and synthesis of cII protein: role of the viral cIII and host *hflA, himA,* and *himD* genes. Cell, *31:* 565–573, 1982.
28. Maxam, A. M., and Gilbert, W. A new method for sequencing DNA. Proc. Natl. Acad. Sci. USA, *74:* 560–564, 1977.
29. Deverreux, J., Haeberli, P., and Smithies, O. A comprehensive set of sequence analysis programs for the VAX. Nucl. Acids Res., *12:* 387–395, 1984.
30. Bell, J. C., Jardine, K., and McBurney, M. W. Lineage-specific transformation following the differentiation of multipotential murine stem cells containing a human oncogene. Mol. Cell. Biol., *6:* 617–625, 1986.
31. Krantz, M. J., Laferté, S., and Ariel, N. Radioimmunoassay of CEA. Methods Enzymol., *84:* 32–48, 1982.
32. Dayhoff, M. O., Hunt, L. T., Barker, W. C., Orcutt, B. C., Yeh, L. S., Chen, H. R., George, D. G., Blomquist, J. C., Fredrickson, J. A., and Johnson, G. C. Protein Sequence Database. Washington, DC: National Biomedical Research Foundation, 1981.