Somatostatin-28 Encoded in a Cloned cDNA Obtained from a Rat Medullary Thyroid Carcinoma*

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We have constructed and cloned in bacteria complementary DNAs derived from a transplantable rat medullary thyroid carcinoma. Using a hybridization probe encoding an anglerfish islet pre-prosomatostatin, a precursor of the tetradecapeptide somatostatin, we have identified and isolated a clone containing a 400-base pair complementary DNA encoding most of the rat carcinoma pre-prosomatostatin. The amino acid sequence of the tetradecapeptide somatostatin and of the amino-terminally extended form, somatostatin-28 was deduced from the nucleotide sequence of the complementary DNA. Somatostatin-28 was found at the COOH terminus of a polypeptide of at least 80 amino acids indicating that somatostatin-28 arises by cleavage from a large precursor. The sequences of somatostatin-28 and somatostatin-14 are strictly conserved between the rat and other mammals. Such conservation of these sequences indicates strong selective pressures during evolution to maintain the sequence and suggests that somatostatin-28 may serve some essential biologic functions apart from, or in addition to, the important regulatory actions of somatostatin-14. Additionally, we found a high degree of homology in the amino acid sequences of the NH2-terminal extension peptides in the anglerfish islet and the rat carcinoma pre-prosomatostatins pointing further to a possible biologic function of these extension peptides.

Somatostatin is a tetradecapeptide that regulates the release of pituitary, pancreatic, and gastrointestinal hormones (1). Initially identified in the hypothalamus as an inhibitor of growth hormone secretion (2), somatostatin has subsequently been found in extrahypothalamic brain, spinal cord, retina, gastrointestinal tract, pancreatic islets, and thyroid (3–6). In addition to inhibiting the secretion of a number of peptide hormones, somatostatin has been proposed to act as a neurotransmitter and to modulate gastrointestinal motility (7, 8).

The diverse functions and the widespread distribution of the tetradecapeptide somatostatin (somatostatin-14) have focused attention on the biosynthesis of the hormone. Several studies have shown that somatostatin-14 is synthesized as part of a larger precursor. A 28-amino acid form of the hormone (somatostatin-28) has recently been identified in extracts of porcine hypothalamus (9), gastrointestinal tract (10), and ovine hypothalamus (11). Somatostatin-28 may have functions distinct from those of the tetradecapeptide (12–14).

Pulse and pulse-chase labeling studies in brain and pancreatic islets indicate that somatostatin-28 is also derived from a larger precursor (15, 16). Cell-free translations of mRNA isolated from the pancreatic islets and gastrointestinal tissues of anglerfish (17–19), the pancreatic islets of channel catfish (20) and rat hypothalami (21) have confirmed the existence of large (Mr, 14,000 to 16,000) precursors of somatostatin (pre-prosomatostatins). Recently, nucleotide sequencing of cloned complementary DNAs (cDNAs) have provided the amino acid sequences of two anglerfish islet pre-prosomatostatins (22, 23). These sequences show that the somatostatin-14 precursors are located at the COOH termini of 119 to 121 amino acid precursors and that the fish and mammalian somatostatin-14 peptides have identical sequences. However, only partial conservation of the somatostatin-28 sequence was observed between fish and mammals. Little is known about the structures of the peptides that lie NH2-terminal to the somatostatin-28 sequence of mammalian pre-prosomatostatins.

We now report the use of a cloned cDNA containing coding sequences for an anglerfish islet pre-prosomatostatin as a hybridization probe to identify a cloned somatostatin-related cDNA derived from a transplantable rat medullary carcinoma of the thyroid. Nucleotide sequencing of the cDNA revealed the complete sequence of rat somatostatin-28 and the partial sequence of the NH2-terminal precursor extension of the pre-prosomatostatin. We find that the amino acid sequence of somatostatin-14 and somatostatin-28 in the rat are identical with those found in the ovine and porcine species. In addition, comparison of the amino acid sequences of the NH2-terminal extensions encoded in the 5′ regions of the fish and rat cDNAs show considerable homology (53% among nucleotides and 39% among amino acids). The latter observations point to the existence of strong selective pressures to conserve the structures of the NH2-terminal extensions over the 400 million years since fish and rat diverged in evolution. These findings suggest that these peptide extensions may have some, as yet unrecognized, biologic function other than simply to serve as protein “spacer” sequences.

EXPERIMENTAL PROCEDURES

Construction and Cloning of cDNAs from a Rat Medullary Carcinoma of the Thyroid—The preparation of a cloned cDNA library from rat medullary carcinoma of the thyroid kindly provided by N. H. Bell was described previously (24). In brief, cDNA was prepared from the polyadenylated RNA (25) by using an oligo(dT) primer and reverse transcriptase (26). Double-stranded DNA was prepared from the cDNA with polymerase I, inserted into the PstI restriction endonuclease site of the plasmid pBR322 (27), and recombinant plasmids were introduced into Escherichia coli χ1776 by the procedure of Vila Komaroff et al. (27).

Identification of Somatostatin-related Medullary Thyroid Carcinoma cDNA—Approximately 2,000 cloned cDNAs derived from the rat medullary thyroid carcinoma were screened using a modification
of the procedure of Grunstein and Hogness (28). Cloned cDNA encoding anglerfish pre-prosomatostatin (22) was digested with the restriction endonuclease Xma I and Dde I, labeled at the 3'-ends with 32P (22), and electrophoresed on 5% polyacrylamide gels containing Tris/borate/Na,EDTA. A 58-base pair DNA fragment encoding the tetradecapeptide somatostatin was isolated from the gels and used as a hybridization probe.

Bacterial colonies containing recombinant cDNAs from rat medullary thyroid carcinoma were grown on nitrocellulose filters. Bacteria were lysed and the DNA was fixed onto the filters as described by Grunstein and Hogness (28). Filters were prehybridized for 18 h at 68°C in a solution containing 6 x SSC (0.9 M NaCl, 0.09 M sodium citrate), 5 x Denhardt's reagent (0.1% w/v each of bovine serum albumin, polyvinylpyrrolidone, and Ficoll), 0.5% sodium dodecyl sulfate, 5 µg/ml of sonicated denatured salmon sperm and E. coli DNA, and 10% dextran sulfate.

After prehybridization, the buffer was discarded and replaced by an identical solution containing heat-denatured hybridization probe. Filters were allowed to hybridize for 2 h at 68°C and were subsequently washed 10 times at 68°C with a solution containing 5 x SSC, 1 x Denhardt's reagent, and 0.5% sodium dodecyl sulfate. Filters were then washed twice at room temperature in 0.01 x SSC and air-dried. Autoradiograms were prepared using Kodak X-O-Mat film exposed for 2 to 3 days at ~90°C using a Dupont Cronix intensifying screen.

**RESULTS**

Immunoreactive somatostatin has been identified in extracts of transplanted rat medullary thyroid carcinomas (30, 31). Preliminary studies using immunoprecipitation and hybridization selection techniques were unsuccessful, however, in identifying somatostatin precursors from the cell-free translation products programed by medullary thyroid carcinoma mRNAs. We had previously constructed a cDNA library from a rat medullary thyroid carcinoma for the purpose of isolating and sequencing cDNA encoding a precursor of calcitonin (24). By using a cloned cDNA encoding a somatostatin precursor from anglerfish islets we were able to identify a rat somatostatin-related cDNA in the carcinoma cDNA library by the colony hybridization method. Only one clone, 400 base pairs in length, was identified out of approximately 2,000 clones which were screened. The paucity of clones containing somatostatin-related cDNAs reflects the low levels of somatostatin found in these tumors (30).

The identification of sites which are cleaved by the restriction endonucleases Xma I, Pst I, and Rsa I proved particularly useful in determining the nucleotide sequence of the cDNA. Restriction fragments were sequenced on both the sense and nonsense strands (Fig. 1).

The nucleotide sequence of the cloned cDNA and the corresponding amino acid sequence is shown in Fig. 2. This cDNA encodes the sequence of the tetradecapeptide somatostatin and somatostatin-28 in addition to a 51-amino acid NH2-terminal extension. As predicted by Patzelt et al. (32), the tetradecapeptide somatostatin is located at the COOH terminus of the precursor, followed by a stop codon TAG. The 14 amino acids predicted by the nucleotide sequence which precede the tetradecapeptide are identical with those found in ovine and porcine somatostatin-28 (9–11).

Somewhat surprising was the nucleotide sequence coding for Leu-Gln-Arg which separates somatostatin-28 from the remainder of the precursor (Figs. 2 and 3). This sequence is reminiscent of the sequence Leu-Glu-Arg found at the analogous position in the anglerfish pre-prosomatostatins (23). In

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1 R. H. Goodman, unpublished data.
asmuch as the sites of cleavages typically found in prohor-
mones consist of combinations of two of the three basic
amino acids arginine and lysine (33), our sequence raises
the question of whether somatostatin-28 is produced by post-
translational cleavages of prosomatostatin in the rat medul-
lar thyroid carcinoma.

The sequence Asn-Gln-Thr within the NH2-terminal exten-
sion of the rat somatostatin precursors represents a potential
N-glycosylation site (34). Patzel et al. have suggested that
rat prosomatostatin may be glycosylated (32). No analogous
potential glycosylation sites are present within the two an-
glerfish pre-prosomatostatins.

Inasmuch as there were differences between the sequences
of anglerfish pre-prosomatostatin I reported by our laboratory
(22) and that of Hobart and co-workers (23), we have exten-
sively reanalyzed the sequence of the anglerfish somatostatin
precursor. The revised sequence, which is slightly different
from either of the two sequences reported previously is shown
in Fig. 4.

Fig. 4 additionally compares the nucleotide and amino acid
sequences of the anglerfish and rat somatostatin precursors.
Within the coding sequence for the tetradecapeptide soma-
tostatin, 36 of the 42 nucleotides (85%) are conserved between
rat and anglerfish. Within the coding sequence for somato-
statin-22, 26 of the 28 amino acids (79%), and 58 of the 84
nucleotides (69%) are maintained. The six amino acid substi-
tutions between the fish and rat somatostatin-28 sequences
appear to be conservative in nature. It is necessary to add or
delete specific codons to maintain the homology between the
NH2-terminal extensions of the fish and rat sequences. If these
deletions and additions are made, 20 of the 51 amino acids
within the extension (39%) are strictly conserved and an
additional 15 amino acid changes are conservative in nature.
Eighty-one of 153 nucleotides (53%) within this region are
conserved. With the exception of the AATAAA sequence and
the polyadenylate tail, there appears to be little conservation
of nucleotide sequence within the 3' untranslated regions.

**DISCUSSION**

A cloned cDNA of 400 base pairs coding for pre-prosoma-
tostatin, a precursor of rat somatostatin, was identified by
colony hybridization using a 32P-labeled cDNA encoding an
angler fish islet pre-prosomatostatin. Previous studies have
indicated that rat hypothalamic and pancreatic somatostatin-
14 are identical in amino acid composition and chromatog-
previously been determined (9-11). Recent evidence suggests that the biologic activities of somatostatin-28 may be greater than, and perhaps different from, those of somatostatin-14 (12-14). Strict conservation of the amino acid sequence of somatostatin-28 between the rat and these other mammals, species which diverged over 75 million years ago (39), is strong evidence for the existence of evolutionary pressures to maintain this sequence. It is likely, therefore, that somatostatin-28 has an important biologic function in mammals. Conservation between anglerfish and mammals of the six amino acids adjacent to the tetradecapeptide somatostatin suggests a particular importance of this portion of somatostatin-28 (Fig. 5).

Examination of the NH₂-terminal portions of the fish and rat somatostatin precursors reveals several additional regions of considerable homology. This observation raises the possibility that the NH₂-terminal portion of prosomatostatin may also have some specific biologic functions. Comparison of somatostatin cDNAs from other species should be useful in understanding the importance of this region. We are currently synthesizing by chemical methods peptide fragments of the NH₂-terminal region of rat prosomatostatin for the preparation of antisera to be used in studies of intracellular transport, secretion, and potential biologic activity of the precursor region of the prosomatostatin. Such studies should further our understanding of neuropeptide biosynthesis and physiology.

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