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Short sequence-paper

Molecular cloning and tissue-specific expression of a new member of the regenerating protein family, islet neogenesis-associated protein-related protein[1]

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Abstract

Islet neogenesis-associated protein (INGAP) is a protein expressed during islet neogenesis. We have cloned a novel cDNA having a similar sequence to INGAP cDNA. The cDNA encodes 175 amino acids designated INGAP-related protein (INGAPrP). INGAP is expressed in cellophane-wrapped pancreas, but not in normal pancreas, whereas INGAPrP was abundantly expressed in normal pancreas.

Keywords: Islet neogenesis-associated protein-related protein; Islet neogenesis-associated protein; Regenerating protein; Pancreatitis-associated protein

Cellophane wrapping of adult hamster pancreas leads to new islet formation in the absence of an inflammatory response [1]. In the extract from cellophane-wrapped pancreas, growth-promoting activity specific to islet cells was detected as 'ilotropin' [2]. Recently, islet neogenesis-associated protein (INGAP) cDNA was cloned by the differential display technique using mRNA extracted from cellophane-wrapped and non-wrapped hamster pancreas [3]. INGAP is abundantly expressed in acinar cells in cellophane-wrapped pancreas and mitogenic to primary-cultured epithelial cells of pancreatic ducts, but not to a hamster insulinoma tumor cell line [3]. Because duct cell proliferation is a prerequisite for islet neogenesis, INGAP is considered to be a constituent of ilotropin. To obtain mouse INGAP cDNA, we carried out reverse transcriptase-PCR (RT-PCR) with a pair of primers based on the sequence of hamster INGAP cDNA. Instead of mouse INGAP cDNA, unknown cDNA encoding a new member of the Reg family, designated INGAP-related protein (INGAPrP), was amplified and cloned. Here we report its nucleotide and deduced amino acid sequences and its tissue-specific expression.

Because INGAP is expressed in the duodenum [3],

Abbreviations: INGAP, islet neogenesis-associated protein; INGAPrP, INGAP-related protein; PAP, pancreatitis-associated protein; Reg, regenerating protein; RT, reverse transcriptase

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[1] The sequence data reported in this paper have been deposited to DDBJ/EMBL/Genbank databases under the accession no. AB028625.

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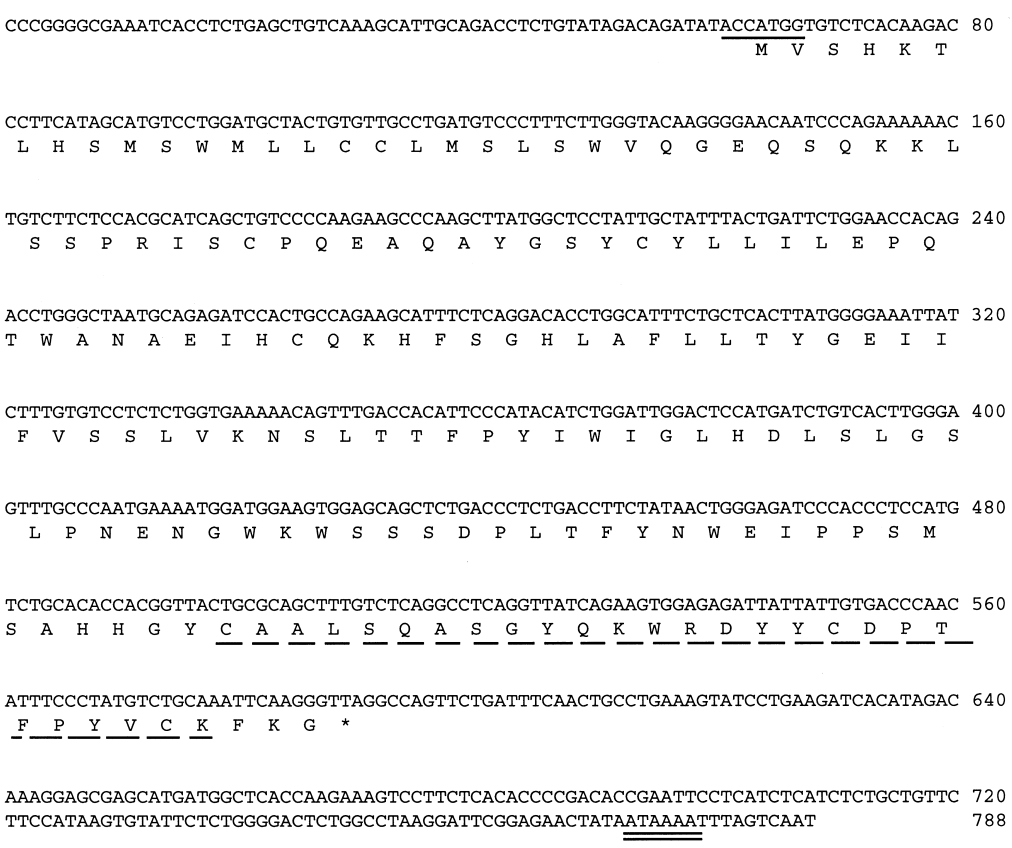

Fig. 1. A compelte cDNA sequence of INGAPrP and a deduced amino acid sequence. A Kozak's sequence around the initiation codon, ATG, is underlined. * indicates a stop codon. A polyadenylation signal is double underlined. A dashed underline indicates a C-type lectin motif detected with a computer program, MOTIF: Searching Protein and Nucleic Acid Sequence Motifs available at http://www.motif.genome.ad.jp.

5 μg of total RNA extracted from mouse duodenum were reverse-transcribed to cDNA with a SuperScript II first strand synthesis kit (Gibco-BRL, Rockville, MD). Using the first-strand cDNA as a template, PCR was performed with oligonucleotide primers specific to the sequence of hamster INGAP cDNA (sense, 5′-CTGCCTTCTTCACGTATAAC-3′; and antisense, 5′-ACTGCACAATAACCACGGTC-3′) and 0.05 U/μl of Ampli Taq Gold (PE Biosystems, Foster City, CA). After the initial denaturation at 94°C for 9 min, one cycle of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min, was repeated 35 times. After 8% polyacrylamide gel electrophoresis, PCR product (approximately 300 bp) was purified by the 'crush-and-soak' method, and subcloned into pCRII vector with a TA cloning kit (Invitrogen, San Diego, CA). Nucleotide sequence was determined using dye terminator cycle sequencing with a Model 377 DNA sequencer (PE Biosystems). To obtain the full cDNA from the sequence of the PCR product, we searched its homologous cDNA clone using DDBJ search system (http://www.ddbj.nig.ac.jp/E-mail/homology.html), and found that EST clone with accession no. AA822059 from *Mus musculus* diaphragm cDNA library had homology of 98%. This EST clone was purchased from Genome Systems (St. Louis, MO), sequenced with T3 or T7 primer, and ascertained to have the identical sequence to the PCR product we obtained. From the homology to hamster INGAP cDNA and the presence of a complete Kozak's sequence [4] with ACCATGG around the first ATG, the coding nucleotide sequence and deduced amino acid sequence were determined (Fig. 1). The NH_2-terminus of the amino acid sequence is highly hydrophobic, and probably functions as a signal peptide to secrete this protein. Computer analysis reveals that the preprotein with a signal peptide has a molecular weight of 20 020.

The deduced amino acid sequence is 72.2% identical to the hamster INGAP (Fig. 2). To examine whether it is a new member of the Reg (regenerating protein) family or mouse INGAP, tissue-specific expression pattern was examined by Northern blot analysis. Total RNA (10 μg) isolated from mouse brain, duodenum, heart, kidney, liver, lung, skeletal muscle, stomach, spleen, and pancreas using Isogen (Nippongene, Tokyo, Japan) was electrophoresed on a 1.0% agarose gel with 2% formaldehyde, transferred onto a nylon membrane, GeneScreen Plus (NEN Research Products, Boston, MA), by capillary transfer, and fixed on it with a UV cross-linker. The *Eco*RI–*Xho*I fragment including the entire cDNA was purified with a Geneclean II kit (BIO 101, La Jolla, CA) after electrophoresis, radiolabeled with [α-^{32}P]dCTP (3000 Ci/mmol, Amersham, Buckinghamshire, UK) using a Megaprime DNA labeling system (Amersham), and used as a probe for Northern blot analysis. Hybridization was carried out at 42°C in 5×SSPE containing 50% formamide, 0.1% SDS, 100 μg/ml salmon sperm DNA, and a radiolabeled probe. mRNA expression was abundant in stomach, duodenum, and pancreas, and modest in skeletal muscle, while no expression was detected in brain, heart, kidney, liver, lung, or spleen (Fig. 3). On the other hand, INGAP mRNA was expressed only in duodenum among non-wrapped organs [3]. Based on the different tissue-specific expression patterns, we found it difficult to designate INGAP-like protein

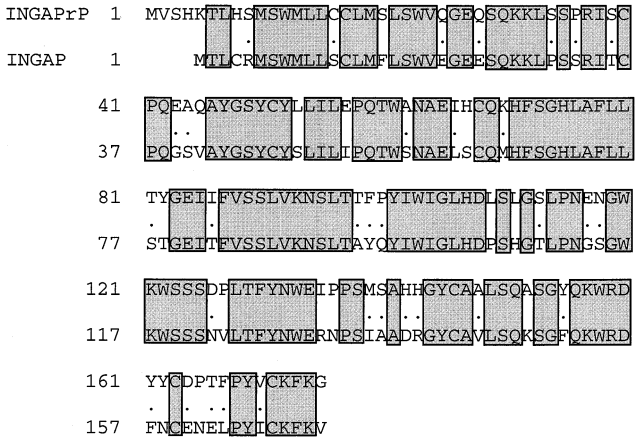

Fig. 2. Comparison of amino acid sequences between INGAPrP and INGAP. There is 72.2% homology in the whole sequence. Shaded areas indicate identical amino acids.

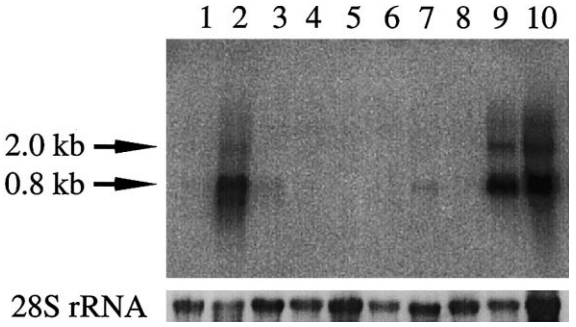

Fig. 3. Northern blot analysis of INGAPrP. Because the upper faint bands (2.0 kb) were not reproducibly found (data not shown) and the alternative splicing of Reg family genes has not been reported, they are regarded non-specific bands. Lane 1, brain; lane 2, duodenum; lane 3, heart; lane 4, kidney; lane 5, liver; lane 6, lung; lane 7, skeletal muscle; lane 8, spleen; lane 9, stomach; and lane 10, pancreas.

cDNA we obtained as mouse INGAP, and newly designated as INGAPrP.

Amino acid sequence of INGAPrP was compared to those of other members of the Reg family including Reg proteins, pancreatitis-associated proteins (PAP), and INGAP (Fig. 4), and showed a 72.2% similarity to hamster INGAP, 52.0% to mouse Reg IIIβ [5], 51.4% to rat PAP-I [6], 50.6% to mouse PAP-II/Reg IIIα [5], 49.7% to mouse Reg IIIγ [5], 49.1% to human PAP-H/HIP [7], and 47.4% to rat PAP-III [8]. All members of the Reg family have a high degree of homology with a consensus motif of the calcium-dependent (C-type) animal lectin, and four conserved cysteines in this motif form two disulfide bonds [9]. The knowledge of functions of lectins and other members of the Reg family are suggestive of functions of INGAPrP. In the insect, C-type animal lectin contributes to the development of some organs in an autocrine manner [10], and some lectins, such as concanavalin A and phytohemagglutinin, are mitogenic for lymphocytes. The Reg I expression level in islets is positively correlated with islet-cell replication [11,12] and negatively associated with islet differentiation [12,14]. Reg I (human pancreatic thread protein) shows a mitogenic activity to both RIN (β) cells and rat pancreas-derived ductal cell line of ARIP [13]. Moreover, the intraperitoneal administration of recombinant rat Reg I protein ameliorates the surgical diabetes due to 90% pancreatectomy, with an increased β-cell mass [15]. Recently, it was reported that Reg II is expressed in developing

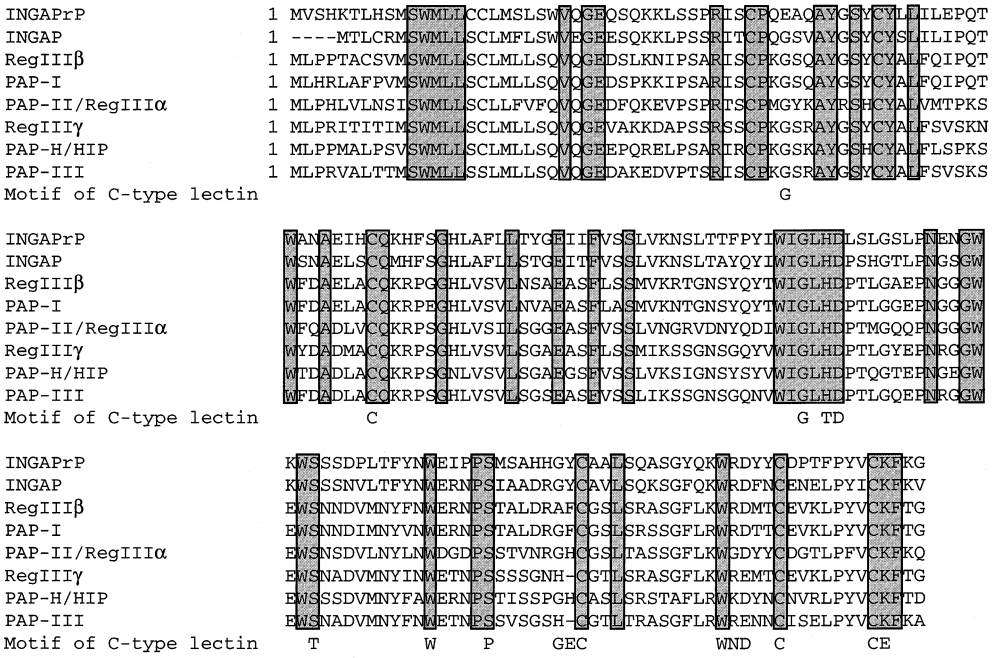

Fig. 4. Alignment of the amino acid sequence of mouse INGAPrP with those of hamster INGAP [3], mouse Reg IIIβ [5], rat PAP-I [6], mouse PAP-II/Reg IIIα, Reg IIIγ [5], human PAP-H/HIP [7], and rat PAP-III [8]. Shaded areas indicate the conserved residues among these eight.

and regenerating motor neurons, and that Reg II produced by regenerating neurons functions as a Schwann-cell mitogen for successful regeneration [16]. Like Reg II, INGAP shows a mitogenic activity to hamster duct epithelia in primary culture and rat pancreatic duct cells, but has no effect on a hamster insulinoma tumor cell line [3]. Taken together, Reg and INGAP seem to play a role in the maintenance of β-cell mass, especially islet neogenesis. Therefore, INGAPrP may also function to regain the decrease in β-cell mass.

Expression patterns of the Reg family are relatively tissue-specific. Except for the expression in the pancreas, Reg I is expressed in stomach [17,18], Reg II in stomach and regenerating neurons [16], INGAP in duodenum [3], and INGAPrP in stomach, duodenum, and skeletal muscle. INGAP is expressed in cellophane-wrapped pancreas, but not in non-wrapped pancreas [3]. In contrast to INGAP, INGAPrP was expressed in non-wrapped normal pancreas. We also examined the effect of streptozotocin on the expression level of INGAPrP by Northern blot analysis, but no change could be detected (data not shown).

Although tissue-specific expression pattern of mouse INGAPrP is considerably different from that of hamster INGAP, there remains a small possibility that INGAPrP is a mouse counterpart of hamster INGAP. Tissue-specific expression pattern of INGAP may vary among individual species. To clearly distinguish INGAPrP from INGAP, we are trying to clone mouse INGAP cDNA from cellophane-wrapped murine pancreas. Further studies on INGAPrP are also underway in our laboratory.

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