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Expression of *Reg* gene in the Syrian golden hamster pancreatic islet regeneration model

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Summary We have reported previously that cellophane wrapping of the hamster pancreas is a stimulus that leads to the induction of duct epithelial cell proliferation, followed by endocrine cell differentiation and new islet formation. *Reg* is a candidate gene that has been reported to be expressed in regenerating pancreatic islets, suggesting a role in islet growth. We examined *Reg* gene expression in the cellophane-wrap model by isolating total RNA from hamster pancreata at various times after wrapping. Northern blot analysis using a rat cDNA *Reg* probe showed no expression of *Reg* in control non-wrapped hamster pancreas, whereas a strong signal was detected in control wrapped rat pancreas. Using reverse transcription of RNA followed by polymerase chain reaction (PCR) we amplified, isolated and sequenced a 194 base pair product which showed homology to rat *Reg* in both control and wrapped hamster pancreas. When the PCR product was used as a probe for Northern blot analysis, no signal was detected in control non-wrapped pancreata. In contrast, a strong signal was detected 1 and 2 days after wrapping, which then returned to basal between 4 and 6 days after wrapping. A similar temporal pattern was observed using *in situ* hybridization to localize the *Reg* gene. One- and 2-day wrapped but not control pancreas expressed *Reg* in acinar cells, but not in islets. In conclusion, (a) *Reg* expression is low in the control hamster pancreas; (b) in the cellophane-wrap model of islet neogenesis, increased *Reg* mRNA is found within acinar tissue; (c) *Reg* gene may thus be involved as an acinar paracrine effector of duct cell proliferation in the initial step of islet neogenesis, but may also participate in the inflammatory response to traumatic stimuli. [Diabetologia (1995) 38: 906–913]

Key words *Reg* gene, islet neogenesis, cellophane wrap, hamster.

In 1982, we developed a model for islet regeneration without tumour formation in hamsters [1]. Cellophane wrapping of the hamster pancreas led to the induction of new islet formation within 2 weeks [2, 3]. These new endocrine cells appear to derive from ductal epithelium elements [4, 5]. Electron microscopic and immunocytochemical examination of the newly-developed islets identified cells containing insulin, glucagon and somatostatin [3] and wrapping was capable of reversing streptozotocin (STZ) induced diabetes [4, 6]. The mechanism whereby cellophane wrapping induces cell proliferation and differentiation is unknown but may be related to induction of local growth factors.

Several studies have attempted to address the role of various factors regulating islet neogenesis. One such factor is *Reg* gene and its protein product; the findings however, have been controversial. Screening of a cDNA library derived from regenerating rat

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Abbreviations: PCR, Polymerase chain reaction; RT, reverse transcription, MOPS, 3-[N-morpholino]propane-sulphonic acid; PSP, pancreatic stone protein; PTP, pancreatic thread protein; PAP, pancreatitis-associated protein; HGF/SF, hepatocyte growth factor/scatter factor; PCR, polymerase chain reaction; STZ, Streptozotocin; PBS, phosphate buffered saline; bp, base pair.

islets after partial pancreatectomy and nicotinamide administration revealed a novel single-copy gene encoding a 165 amino acid protein, which has been named *Reg* gene [7, 8]. Terazono et al. [9] reported the expression of *Reg* protein both in rat acinar cells and co-localized with insulin in the beta cells. The *Reg* gene was found to be identical to an exocrine pancreatic gene named pancreatic stone protein (PSP) [10], or pancreatic thread protein (PTP) [11] whose putative function is to inhibit calcium lithiasis in the exocrine pancreas [12]. Smith and colleagues, using 90 % pancreatectomized rats showed increased *Reg* gene expression 1–3 days after pancreatectomy that correlated with growth of both exocrine and endocrine tissue [13]. However, they reported a lower increase in *Reg* gene expression in their sham-operated rats and under other conditions that were not associated with pancreatic beta-cell growth [13]. Others have shown that isolated rat islets accumulate thymidine in parallel with increased *Reg* gene expression in response to a variety of growth factors [14]. Recently, *Reg* protein was shown to stimulate pancreatic beta-cell growth [15] and Otonkoski et al. [16] demonstrated increased *Reg* expression in hepatocyte growth factor/scatter factor (HGF/SF) induced proliferation of beta cells [16]. As a corollary, Miyaura and colleagues showed that implantation of an insulinoma into New England Deaconess Hospital (NEDH) rats caused a sharp reduction in *Reg* expression associated with a profound reduction in non-tumour islet size relative to normal controls. Removal of the tumour resulted in rapid beta-cell proliferation and a large but transient increase in *Reg* mRNA levels in non-islet tissue [18]. Based upon these observations it was suggested that *Reg* may be expressed in acinar tissue and not in regenerating islets, but nonetheless may have been implicated in the regenerative process.

Confounding the problem of interpreting the findings with *Reg* is the recent information that there are several *Reg*-related genes and proteins with structural homology. *Reg* II was described in the mouse pancreas and hyperplastic islets [19], pancreatitis-associated protein (PAP) I, II and III were described in the rat and human during acute pancreatitis [20–23], and a novel gene named HIP was described in liver cancer [24].

In light of the possible role of *Reg* in islet neogenesis we elected to study the *Reg* gene expression in our model of islet regeneration. These studies show, in our hamster model of regeneration, that *Reg* gene expression precedes the islet regenerative response and occurs as shown by *in situ* hybridization in peri-islet acinar tissue compatible with a paracrine role of *Reg* protein. These studies extend the scope of our understanding of the role of *Reg* gene as a local regulator of islet regeneration to another species and identify a possible role for *Reg* gene in new islet formation in the adult pancreas. We also provide clear delineation between hamster *Reg* and PAP.

Materials and methods

Animals. Thirty outbred female Syrian golden hamsters, 7 weeks of age (Charles River, Kingston, New York, USA) were used. All the experiments were conducted in accordance with the principles and procedures outlined in the NIH "Guide for the Care and Use of Laboratory Animals". Our model for surgical induction of cell proliferation in the pancreas of the Syrian golden hamster has been described previously [1, 2]. Briefly, a midline laparotomy incision is made. With the aid of a stereo dissecting microscope, the distal common bile duct and head of the pancreas are exposed. Using blunt dissection, an avascular plane is developed to allow the placement of a 2-mm wide strip of sterile cellophane tape (Imperial Tobacco, Montreal, Canada), which is wrapped around the head of the gland and tied loosely in position.

To study *Reg* gene expression in our model required measurement of total changes in *Reg* mRNA using Northern analysis with a cDNA probe and *in situ* hybridization using an RNA probe. However, since preliminary studies had indicated the uniqueness of hamster *Reg*, this required synthesis of our own probe.

Northern blot hybridization analysis. For Northern analysis, total cellular RNA was isolated from the hamster pancreas using the Chamczynski and Sacchi, guanidine isothiocyanate method [25], which is specifically formulated for tissues with high RNase contents.

Denatured total RNA (30 μg) was separated on a 1.2 % agarose, 0.6 % formaldehyde/MOPS denaturing gel [26], transferred to nylon membrane (Nytran, Schleicher & Schuell, Keene, N.H., USA) by capillary method, and immobilized by baking at 80 °C for 1 h. The cDNA probes used in Northern hybridizations were labelled by incorporation of [α^{32}P]-dCTP (Dupont-New England Nuclear, Boston, Mass., USA) using random primed synthesis [27] to a specific activity of greater than 1×10^9 cpm/μg. The 194 base pair (bp) hamster pancreas polymerase chain reaction (PCR) generated probe homologous to *Reg* gene was synthesized in our laboratory. A 726 bp rat cDNA probe for *Reg* was kindly provided by Dr. H. Okamoto, Toyama, Japan, chicken β-actin was purchased from Oncor Inc. (Gaithersburg, Md., USA). A [γ^{32}P]-ATP (Dupont-New England Nuclear) 5′end-labelled 24mer synthetic oligonucleotide for the 18S ribosomal mRNA was used as a loading control. Prehybridization and hybridization buffers consisted of 50 % formamide, 5 X SSPE, 1 % SDS, 5 X Denhardt's, and 200 μg/ml denatured salmon sperm DNA. Prehybridizations were performed at 50 °C for 4 h and hybridizations at 50 °C for 16–18 h with $1–5 \times 10^6$ cpm/ml of the appropriate radiolabelled probe. Washes were performed twice for 15 min each in 6 X SSPE 0.1 % SDS at 25 °C; 1 × SSPE 0.1 % SDS at 37 °C; and under stringent conditions in 0.1 X SSPE 0.1 % SDS at 65 °C. Autoradiography was performed at –80 °C for 1 h or 8 days by exposing scientific imaging film (X-OMAT/AR, Kodak, Rochester, N.Y., USA).

Preparation and amplification of hamster cDNA PCR. In order to synthesize a hamster-specific probe we applied PCR using rat-specific primers since the hamster *Reg* gene has not yet been isolated. The primers were selected in a region of high homology between species, anticipating that under our experimental conditions the primers would recognize the hamster

Table 1. Primer pair for amplifying hamster *Reg* cDNA

cDNA	Primers	Size of PCR product (base pair)
Reg	5′CAGATCTTTTTTGCCA-GAACATGA-3′ (176—199) 5′-CCAGGATTTGTAGAGAAA-CAGAG-3′ (346–369)	194

gene. In addition, the primers were selected in a region of divergence between *Reg* and PAP genes to assure that no cross-hybridization would occur during the Northern blot analysis.

cDNA was synthesized from 1 μg total RNA using 50 U Moloney murine leukemia virus (M-MLV) reverse transcriptase, primed with 100 ng random hexamers, in a 20-μl solution of 10 mmol/l Tris-HCl (pH8.3), 50 mmol/l KCl, 5 mmol/l $MgCl_2$, 20 U RNase inhibitor and 0.5 mmol/l each deoxynucleoside triphosphate (dNTP), for 1 h at 42 °C, followed by 5 min at 95 °C to inactivate the RT. The resulting cDNA was amplified using PCR [28] with 0.15 mmol/l primers specific for the *Reg* gene (Table 1) in 100 μl of a solution containing 50 mmol/l KCl, 10 mmol/l Tris-HCl (pH 8.3), 1 mmol/l $MgCl_2$ and 2.5 units of AmpliTaq DNA polymerase. After an initial denaturation at 94 °C for 1 min, the samples were subjected to 40 cycles of amplification at an annealing temperature of 55 °C for 1 min and extension at 72 °C for 1 min. To exclude any amplification product derived from genomic DNA that could contaminate the RNA preparation, we selected primers so that the amplified regions spanned an intron of the gene. The reactions were carried out in a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, Conn., USA). The PCR products were analysed by electrophoresis on a non-denaturing 5 % polyacrylamide gel and silver staining applying a modification of the method used by Budowle et al. [29]. To confirm their identity, the PCR product was cloned into the PCR-II vector using the TA cloning kit from Invitrogen (San Diego, Calif., USA) and sequenced with SP6 and T7 promoter primers using the TaqTrack DNA Sequencing System (Promega, Madison, Wis., USA).

Slot blot analysis. To ensure that the hamster *Reg* probe did not detect PAP mRNA in the Northern blots, PAP cDNA (cloned in our laboratory, unpublished data), and hamster *Reg* cDNA were applied to a nylon membrane in various amounts (0.1–10 μg) using a Minifold II (Schleicher & Schuell) slot blotter apparatus and immobilized by baking at 80 °C for 1 h. The membrane was then hybridized with an α^{32}P-labelled hamster *Reg* probe for 24 h and washed under the same conditions used for the Northern analysis.

In situ hybridization. To determine the localization of *Reg* gene in the hamster pancreas we applied the *in situ* hybridization technique and used RNA probes instead of cDNA. The reason was that RNA forms stronger hybrids with the mRNA than the cDNA probes, thus allowing us to perform the hybridizations and washes under high stringency conditions.

Synthesis of RNA probe. In order to generate sense, antisense and RNA probes of high specific activity, transcription was performed in 40 mmol/l Tris-HCl (pH 7.5), 6 mmol/l $MgCl_2$, 2 mmol/l spermidine, 10 mmol/l NaCl, 10 mmol/l dithiothreitol, 0.5 mmol/l ATP, GTP, and UTP, 5 units of RNasin ribonuclease inhibitor (Promega), 80 μCi [^{35}S]-CTP (850 Ci/mmol, Amersham, Arlington Heights, Ill., USA), 1 μg of linearized 194 bp cDNA encoding hamster *Reg* in PCR-II vector as template, and 5 units of T7 or SP6 RNA polymerase. Transcription was carried out at 42 °C for 1 h and the DNA template was removed by incubation with 1 unit of DNase for 15 min at 37 °C. Radionucleotide incorporation was measured by thin-layer chromatography, yielding typical values in the range of 75–95 % incorporation. This produced 80 to 120ng of probe with a specific activity greater than 1×10^9 dpm/μg. After removal of unincorporated nucleotides, probes were reduced in size by limited alkaline hydrolysis to a size range of about 75–150 bp [30] and stored in a 50 % formamide and 10 mmol/l dithiothreitol solution at –70 °C.

Hybridization. In situ hybridization was performed according to the protocol of Ingham and co-workers [31] with some modifications. Control and wrapped animals were perfused with 4 % paraformaldehyde in phosphate buffered saline (PBS) at 37 °C. The pancreata were dissected out, post-fixed for 1–2 h in the same fixative at 4 °C, cryoprotected in 30 % sucrose in PBS overnight, and mounted in Tissue-Tek (Miles Inc. Elkhart, Ind., USA) at –20 °C for frozen sectioning. Sections (8–10 μm) were cut on a cryostat at –20 °C. Pre-hybridization consisted of the following: Sections were fixed in 4 % paraformaldehyde at room temperature, rinsed in PBS, followed by incubation in 2 X SSC (300 mmol/l NaCl, 30 mmol/l sodium citrate, pH 7.0) at 70 °C for 30 min. Sections were then treated with 10 μg/ml proteinase K in 100 mmol/l Tris (pH 8.0) and 50 mmol/l EDTA for 10 min at 37 °C, post-fixed in 4 % paraformaldehyde and rinsed in PBS. To block positive charges, sections were rinsed in 100 mmol/l triethanolamine pH 8.0 for 3 min, acetylated in the same buffer for 10 min, and dehydrated. Hybridization conditions were performed as described [31], and 5×10^7 cpm of probe in 70 μl of hybridization buffer was applied to the slide. The slides were then incubated for 18 h at 55 °C. Post-hybridization included washing slides in WDTT buffer (50 % formamide, 300 mmol/l NaCl, 10 mmol/l Tris-HCl, 10 mmol/l NaPO4 [pH 6.8], 5 mmol/l EDTA, 1X Denhardt's solution, 10 mmol/l dithiothreitol) at 50°C and treating with RNase A (20 mg/ml) in NTE buffer (500 mmol/l NaCl, 10 mmol/l Tris-HCl [pH 8.0], and 1 mmol/l EDTA) at 37 °C, followed by three NTE washes and an 18 h incubation in WDTT at 50 °C. Slides were then dehydrated, dipped in Kodak NTB2 emulsion, dried, and stored at 4 °C. Following 1–3 weeks of exposure, the slides were developed with Kodak D-19 developer (1:1) dilution for 4 min at 15 °C and fixed in Kodak fixer and stained with haematoxylin and eosin. Photographs were taken under darkfield and brightfield optics.

Results

Northern blot analysis of Reg gene expression in pancreatic tissue from rat and hamster. When pancreatic tissue was excised from control rats and from hamsters at various times after the cellophane wrapping and total RNA was isolated, an abundance of a 900-nucleotide transcript was found in normal rat pancreatic tissue which hybridized to the rat *Reg* probe, but none was detected in hamster pancreatic tissue at 4, 8 and 14 days after the wrapping procedure (Fig. 1). No signal was detected in a hamster insulinoma cell line (HIT cells) either. Despite performing numerous Northern hybridizations under various levels of stringency the only signal we observed with the rat *Reg* probe in the hamster tissue was very low.

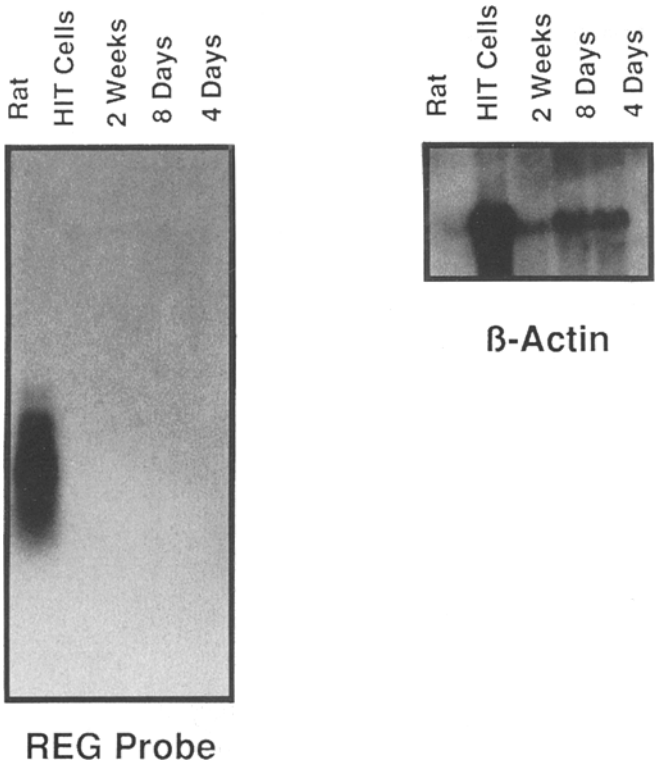


Fig. 1. Northern blot analysis of *Reg* gene expression in pancreatic tissue from cellophane-wrapped hamster, control rat and HIT (hamster insulinoma tumour) cells. Total RNA (30 μg) was separated by electrophoresis on a 1.2 % agarose, 0.6 % formaldehyde/MOPS denaturing gel, and transferred to nylon membrane. Hybridization with rat *Reg* and β-actin cDNA probes were carried out as described in "Materials and methods". Blots were washed and exposed to film (Kodak X-Omat AR) for 48 h at –80 °C

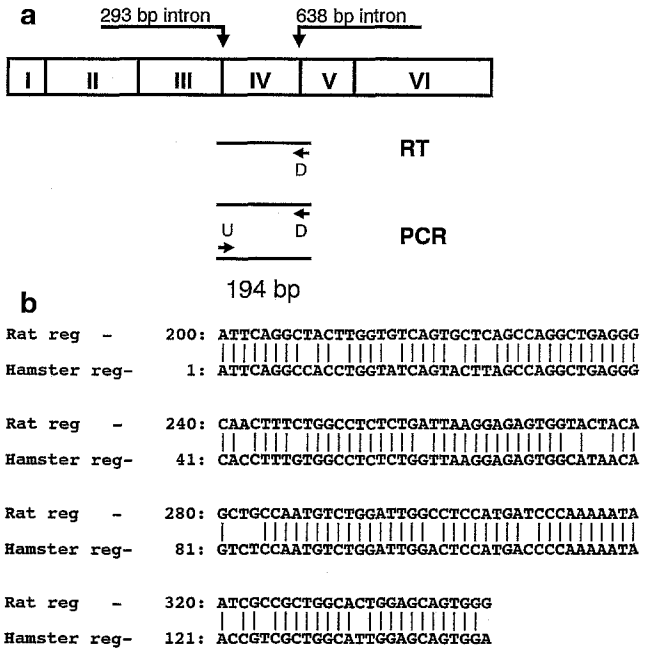
The near-absence of hybridization of the rat cDNA to hamster RNA raised three main questions: (a) was the *Reg* gene in the hamster present only in minute amounts? (b) was the hamster mRNA sequence so different from that in the rat that it avoided detection using the rat cDNA probe? or (c) was *Reg* gene expressed only at specific time points which we did not include initially and thus escaped our detection? To address these questions we: (a) resorted to signal amplification using RT-PCR, (b) isolated, cloned into PCR-II vector and sequenced the PCR product, (c) used the cloned PCR fragment as a hamster-specific probe for Northern blot analysis, (d) extended the time course of wrapping to include early and intermediate time points.

Reg mRNA amplification by PCR. Because it is important to be in the exponential phase of the PCR in order to relatively quantitate the PCR products, we first examined the effect of varying the number of cycles of amplification on the yield of the products corresponding to *Reg*. For *Reg* mRNA, the exponential portion of the curve corresponded to 35–45 cycles. Using *Reg*-specific primers we amplified and isolated a 194 bp product. To exclude any amplification product derived from genomic DNA that could contaminate the RNA preparation, we selected primers such that the amplified regions spanned an intron of the gene (Fig. 2 a). The product was sequenced and identified as a fragment of *Reg* gene with a 20-nucleotide difference compared to the rat *Reg* gene sequence (Fig. 2 b). The PCR product was detected in all the samples tested. Figure 2 c shows the PCR product in control, 1- and 2-day wrapped pancreata.

Northern blot analysis of Reg gene expression in pancreatic tissue from hamster using a hamster-specific probe. To exclude the possibility that failure to detect the *Reg* transcript in the hamster pancreas was due to the inability of the rat probe to detect it, we used the 194 bp PCR product as a hamster specific probe on Northern blot analysis. In addition, in order to determine whether transient expression of *Reg* gene in the hamster pancreas was the cause of our earlier failure to detect it in the previous experiment, we extended the time course of wrapping to include the following 1, 2, 6, 10, 28 and 42 days. No message was detected in control hamster pancreata. However, a strong 900 bp signal was detected 1 and 2 days after wrapping which decreased thereafter (Fig. 3). The size of the transcript was the same as that detected by the rat *Reg* cDNA probe. However, the membrane hybridized with the rat *Reg* cDNA had to be exposed to imaging film for 8 days in order to detect any signal as opposed to 3-h exposure with the hamster probe, in spite of equal specific activity of both probes (not shown). To ensure equal loading of the samples we stripped the blot and used an 18S ribosomal RNA 24mer oligonucleotide probe which hybridized equally to all samples (Fig. 3).

Slot blot analysis of hamster PAP and *Reg* cDNAs showed clearly that the hamster *Reg* probe does not detect PAP (not shown). Hence, it is unlikely that the hamster *Reg* probe detected PAP mRNA in our Northern blot analysis data.

Localization of Reg mRNA by in situ hybridization. When *in situ* hybridization was performed on specimens from 1-, 2- and 10-day wrapped and control non-wrapped pancreata, in the control animals we observed no expression of *Reg* mRNA in pancreatic ductal and acinar cells nor did we find any hybridization in the pancreatic islets (Fig. 4A, B). In contrast, tissue sections from 1-day wrapped pancreata showed strong hybridization with *Reg* mRNA in acinar cells (Fig. 5A, B), in particular in the vicinity of a duct (Fig. 5C). No hybridization, however, was detected in the pancreatic islets (Fig. 5A, B). Tissue sections from 2-day wrapped pancreata showed the same pattern as those from 1 day. The corresponding tissues hybridized with a sense probe showed negligible hybridization (not shown). Sections from 10-day



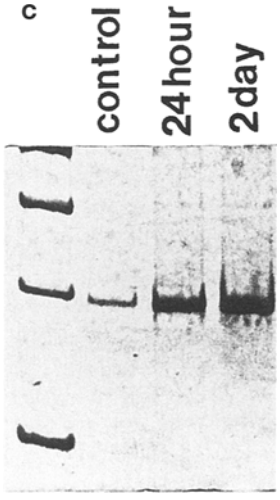


Fig. 2. **a** Schematic structure of *Reg* mRNA and RT-PCR strategy. The coding domains of *Reg* mRNA in the mammalian genome are indicated as I–VI and the positions of two introns in the III and in the IV domains (based on the rat sequence published by Miyashita et al. in the EMBL data base) are also indicated. The upstream (U) and downstream (D) primers (Table 1) were selected such that the amplified region spans two introns of the gene to exclude any amplification product derived from genomic DNA which could contaminate the RNA preparation. **b** Nucleic acid sequence comparison between the hamster PCR product and rat *Reg* gene. RT-PCR was performed using 1 μg of total RNA under conditions described in "Methods", using *Reg*-specific primers. The amplified 194 bp product was cloned into the PCR-II vector and sequenced with SP6 and T7 promoter primers as described in "Methods". The PCR product was compared to the rat *Reg* sequence published by Terazono et al. [9]. A difference of 20 nucleotide was detected between the hamster and the rat *Reg* genes in the area examined. **c** Silver stained polyacrylamide gel showing *Reg* gene expression in whole pancreas in control non-wrapped animals and after 1 and 2 days of wrapping. Aliquots of the amplified products were fractionated on a 5% polyacrylamide gel and silver stained

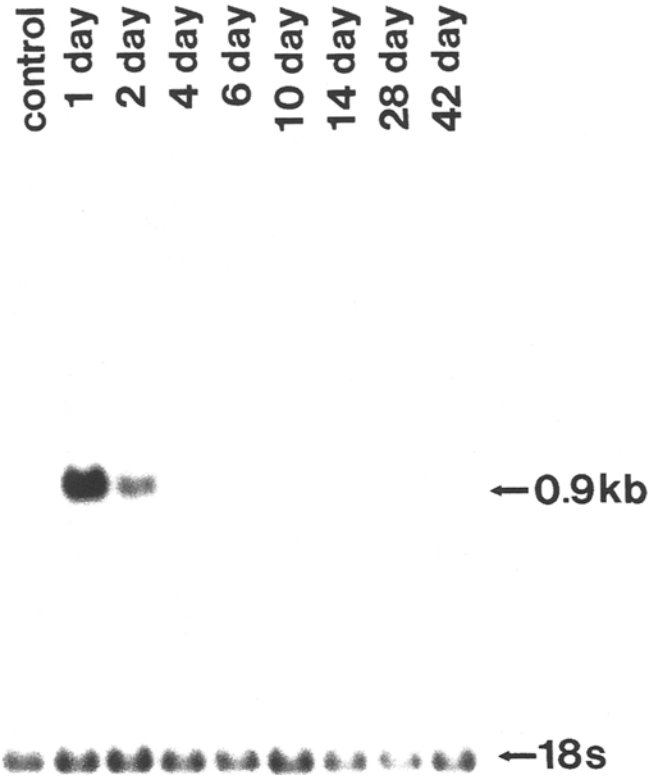


Fig. 3. Northern blot analysis of *Reg* gene expression in pancreatic tissue from control and wrapped hamster pancreas. 30 μg of heat denatured total RNA was separated by electrophoresis on a 1.2% agarose, 0.6% formaldehyde/MOPS denaturing gel, and transferred to nylon membrane. Hybridizations with a hamster-specific cDNA probe generated by PCR and with an 18 s ribosomal 24mer synthetic oligonucleotide probe were carried out as described in "Materials and methods." Blots were washed and exposed to film (Kodak X-Omat AR) for 3 h at –80 °C

wrapped pancreata showed no hybridization with *Reg* riboprobe (data not shown), the same as in control pancreata.

Discussion

In the current study, we examined *Reg* gene expression in a model wherein surgical wrapping of the head of the pancreas with cellophane in the Syrian golden hamster, results in the induction of growth, proliferation of a proto-undifferentiated cell, and differentiation of new pancreatic islet tissue from ductal cells [1–3]. The new islets form randomly in a non-uniform manner that is identical to the dynamics underlying normal islet formation during embryogenesis [4]. Over the ensuing 6 weeks, the small areas of neoislet formation enlarge and assume a size commensurate with the animal's age [2, 32]. It was our expectation that if *Reg* was important in islet regeneration in the adult hamster pancreas, it would have been expressed early in the regenerative response,

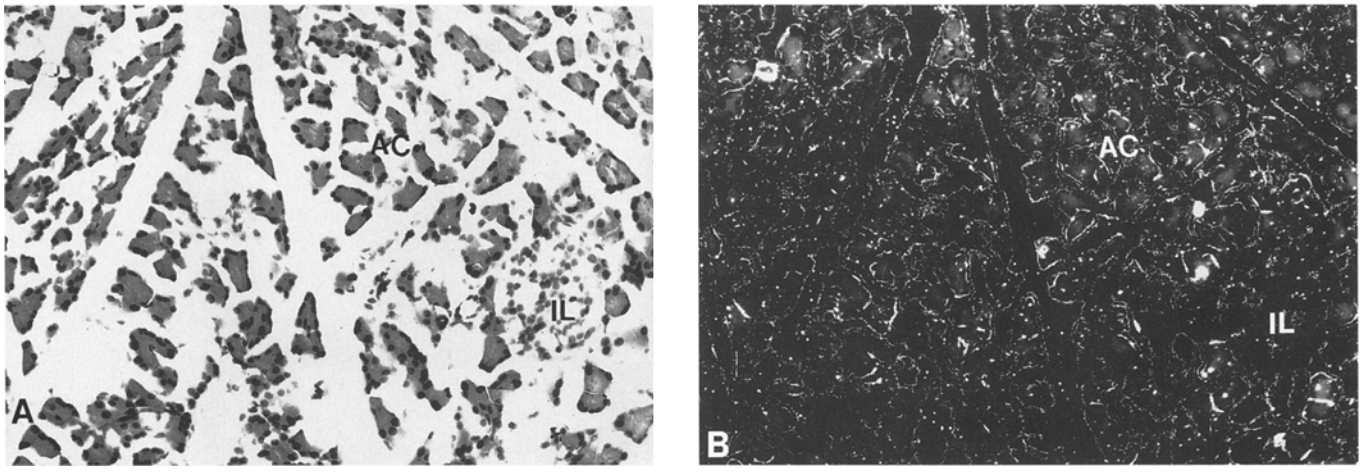


Fig. 4A, B. Localization of *Reg* mRNA by in situ hybridization in control pancreas. Cryostat sections from control pancreas (**A, B**) were hybridized with ^{35}S-labelled antisense single stranded *Reg* RNA probe transcribed from a 194 bp hamster cDNA. **A, B.** Bright and dark-field photographs of pancreatic acinar cells (AC) and an islet (IL) showing no expression of *Reg* mRNA

but we were surprised to find no expression using a rat cDNA probe. Failure to detect *Reg* mRNA by Northern hybridization using rat cDNA was due to species differences and heterology between the rat probe and the hamster gene. The technique was clearly adequate since we found high levels of *Reg* mRNA in the normal rat pancreas, which was in agreement with findings reported by Miyaura et al. [17]. The differences in the sequence are not large since sequencing the 194 bp product revealed a 13.7 % difference between the hamster and the rat in the 146 bp studied (the 194 bp PCR product includes both primers used for amplification). Using the PCR product as a probe in Northern blot analysis showed an undetectable basal level in control animals followed by an immediate increase in *Reg* gene expression within 1 to 2 days after wrapping which decreased thereafter and returned to control level after 4 days.

Using *in situ* hybridization, we observed that all of the *Reg* mRNA was localized in acinar tissue without expression in the islets. In control non-wrapped pancreata, no *Reg* mRNA was detected in any area analysed. Terazono et al.[8] reported increased expression of *Reg* mRNA in two models of islet regeneration, 90 % pancreatectomized, aurothioglucose-injected mice [7] and nicotinamide-treated rats. The *Reg* mRNA was shown to be markedly elevated in islets induced to proliferate by these procedures [7, 8]. In a different model, Miyaura et al. [17] demonstrated that insulinoma implantation in NEDH rats caused a sharp reduction in the normally high expression of *Reg* gene in the exocrine pancreas. This reduction was associated with a profound reduction in beta-cell mass. Resection of tumours resulted in a large but transient induction in *Reg* mRNA levels in the exocrine tissue, associated with beta-cell proliferation. Smith et al. [13] reported a rapid induction of *Reg* expression in the exocrine pancreatic tissue within 24 h after sham or partial pancreatectomy and a return to normal levels by 7 to 14 days, which did not correlate with pancreatic or beta-cell growth. Our data show that induction of islet neogenesis in the hamsters is associated with a rapid rise in *Reg* expression in the acinar tissue but not in islets. The findings that *Reg* expression occurred in exocrine tissue is compatible with its participation in islet neogenesis whether this occurred from islet [15] or from non-islet ductal tissue as we have shown [4]. The absence of *Reg* gene expression in the islets of the wrapped hamster pancreas is consistent with findings by investigators who report expression in exocrine but not in endocrine cell lines [18]. This does not detract from the possible role of *Reg* in stimulating the ductal derived pleotropic cell to differentiate into an adult islet. It does suggest however, that *Reg* has no role in the mature islet. This contrasts with a recent study by Bone et al. [33] who reported high levels of *Reg* gene in pancreatic biopsies from diabetes-prone BB/S rats at or around the mean age for onset of diabetes (90/100 days), and suggested an association of the *Reg* gene with an activation of islet cell adaptive repair/regeneration mechanisms in response to autoimmune islet cell attack. Other studies which support a direct effect of *Reg* on islets report increased *Reg*/PSP expression in cultured islets after stimulation with various factors [14], stimulation of pancreatic beta-cell growth by administration of *Reg* protein [15] and increased *Reg* expression in fetal beta cells in response to HGF/SF [16]. We have previously reported that regenerating pancreatic tissue contains ilotropin, a factor which upon administration to control adult animals and to STZ-diabetic animals causes islet regeneration [34]. We postulated the presence of a local autocrine or paracrine substance capable of initiating proliferation of a proto-undifferenti-

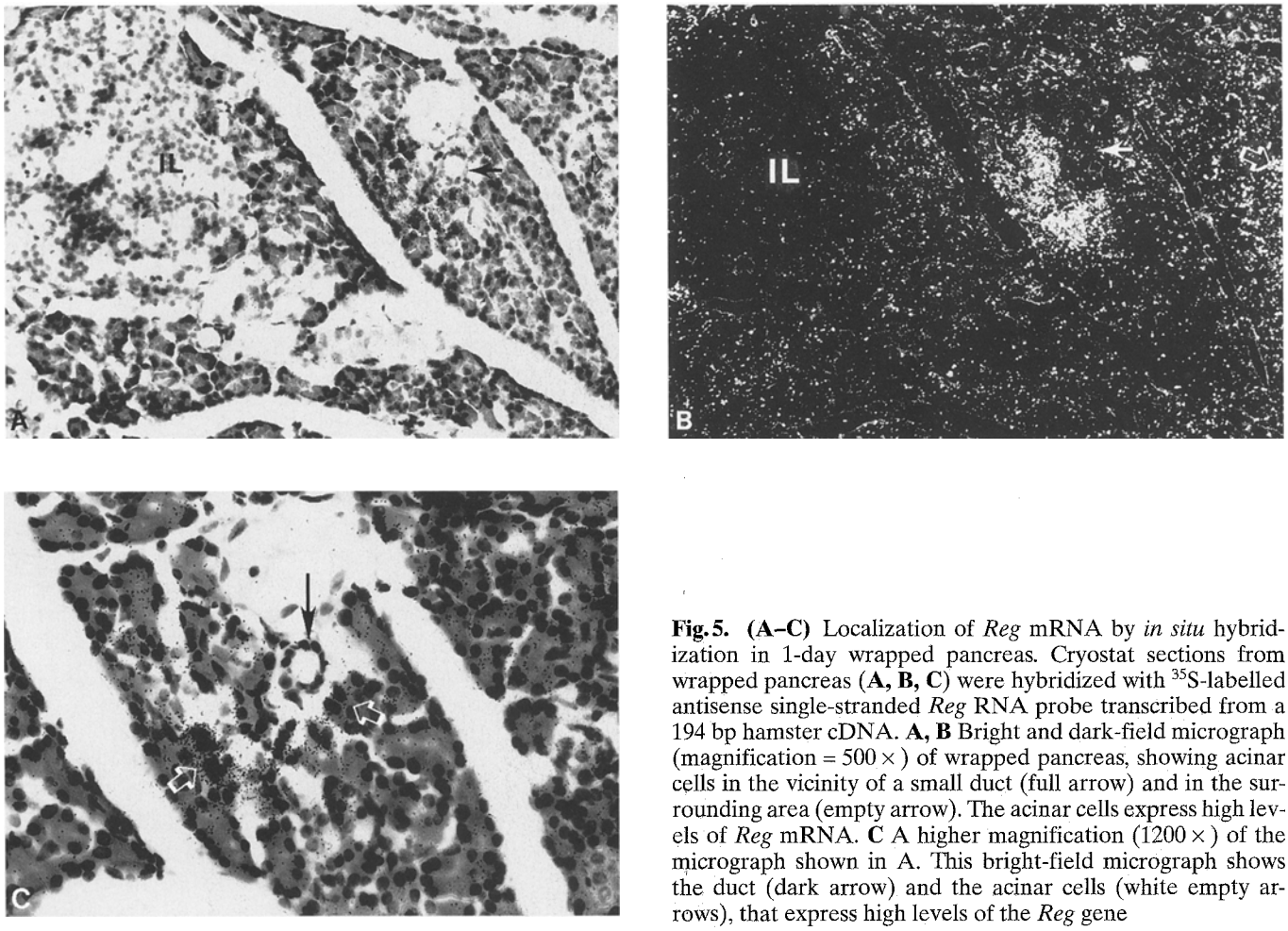


Fig. 5. (A–C) Localization of *Reg* mRNA by *in situ* hybridization in 1-day wrapped pancreas. Cryostat sections from wrapped pancreas (**A, B, C**) were hybridized with ^{35}S-labelled antisense single-stranded *Reg* RNA probe transcribed from a 194 bp hamster cDNA. **A, B** Bright and dark-field micrograph (magnification = 500 ×) of wrapped pancreas, showing acinar cells in the vicinity of a small duct (full arrow) and in the surrounding area (empty arrow). The acinar cells express high levels of *Reg* mRNA. **C** A higher magnification (1200 ×) of the micrograph shown in A. This bright-field micrograph shows the duct (dark arrow) and the acinar cells (white empty arrows), that express high levels of the *Reg* gene

ated cell. Whether ilotropin and *Reg* share similarities remains to be seen.

It has been shown that the *Reg* protein sequence is identical to that of pancreatic stone protein (PSP) [10, 11] and that both are derived from the same gene [35]. The portion of the hamster *Reg* gene we analysed showed homology to the rat *Reg*/PSP gene and seems to be closer to *Reg-I* than *Reg-II* based on amino acid sequence comparison [19]. In addition, several *Reg*-related genes and proteins have been reported recently, defined as *R*eg type III by Unno et al. [19], but known as the pancreatitis-associated protein (PAP) family [20–22]. The hamster *Reg* probe corresponds to a *bona fide Reg*/PSP mRNA and not to a member of the PAP family. The insertion corresponding to amino acids TLGGE of rat PAP in position 104–109 reported by Unno et al. [19] is not present in the hamster *Reg* probe. In lieu of this fact and due to the high stringency conditions of hybridization and washing used in the Northern procedure we believe that it is unlikely for cross-hybridization to occur between Reg/PSP and any member of the PAP family. Moreover, we can completely exclude the possibility that the hamster *Reg* probe detects PAP [20–22] since slot blot analysis in which both hamster PAP cDNA cloned in our laboratory, and hamster *Reg* cDNA did not hybridize with ^{32}P-labelled *Reg* cDNA. However, we cannot completely exclude the possibility that the hamster *Reg* probe might detect other proteins such as HIP [22] since hamster HIP has not been cloned.

Our data thus support a possible role for *Reg*, or a related product in islet neogenesis from a non-islet source. The fact that *Reg* gene expression was only detected in acinar tissue is not supportive of a direct role of *Reg* in the islet per se, but there may be species differences in *Reg* expression. It will require more studies on isolated islets from different species in non-traumatic and non-inflammatory models of neogenesis to resolve these issues.

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