Cell Tissue Res (2003) 312:291–299
DOI 10.1007/s00441-003-0733-1

REGULAR ARTICLE

Severin Meili · Rolf Graf · Aurel Perren · Marc Schiesser · Daniel Bimmler

Secretory apparatus assessed by analysis of pancreatic secretory stress protein expression in a rat model of chronic pancreatitis

Received: 16 December 2002 / Accepted: 4 April 2003 / Published online: 23 May 2003

Abstract Secretory stress proteins (SSP) are a family of proteins including isoforms of pancreatitis-associated protein (PAP) and pancreatic stone protein (PSP/reg). In vitro exposure to trypsin results in the formation of insoluble fibrillar structures. SSP are constitutively secreted into pancreatic juice at low levels. The WBN/Kob rat is a model for chronic pancreatitis, displaying focal inflammation, destruction of the parenchyma and changes in the architecture of the acinar cell; the synthesis and secretion of SSP are also increased. We have investigated the secretory apparatus by SSP immunohistochemistry at the light- and electron-microscopical (EM) levels. Immunocytochemistry of PSP/reg in Wistar control rats reveals low levels, with individual acinar cells exhibiting high immunoreactivity in zymogen granules. PAP is not detectable. In the WBN/Kob rat, PSP/reg and PAP immunoreactivity is markedly increased. Double immunofluorescence for PSP/reg and PAP I or II demonstrates that these proteins colocalize to the same cell. Acinar cells change their secretory architecture by fusion of zymogen granules and elongation of the fused organelles. The immunogold technique has demonstrated an increase of SSP in zymogen granules in WBN/Kob rats. PSP/reg-positive zymogen granules fuse to form elongated structures with fibrillar contents. An extensive PSP/reg-positive fibrillar network is established in the cytosol. Extracellular fibrils have been observed in several ductules. Thus, SSP-derived fibrils form concomitantly with acinar damage in the WBN/Kob rat. Based on the known tryptic cleavage site of SSP, the in vivo generation of fibrils is presumably the result of premature trypsin activation.

Keywords Pancreatitis-associated protein · Pancreatic stone protein · Secretory stress proteins · Pancreas · Rat (WBN/Kob; Wistar; male)

Financial support by the Swiss National Science Foundation (3200–066728.01) and the Amelie Waring Stiftung is greatly appreciated

S. Meili · R. Graf (✉) · M. Schiesser · D. Bimmler
Pancreatitis-Research Laboratory DL 36,
Department of Visceral and Transplant Surgery,
University Hospital Zürich,
8091 Zürich, Switzerland
e-mail: rgf@chi.usz.ch
Tel.: +41-1-2552071
Fax: +41-1-2554393

A. Perren
Department of Pathology,
University Hospital Zürich,
Zürich, Switzerland

Introduction

The male WBN/Kob rat has been established as a model for chronic pancreatitis (Ohashi et al. 1990; Ashizawa et al. 1995). The pancreas of these animals undergoes focal changes of acinar apoptosis, inflammation, acinar-to-duct metaplasia with development of tubular complexes, and fibrosis. Furthermore, inflammatory infiltration appears to affect the cellular architecture of acinar cells: the acini appear rounded and less densely packed and separate from each other. They lose cytosolic contents, particularly zymogen granules. Acinar apoptosis is responsible for parenchymal loss (Hashimoto et al. 2000).

During the period of active inflammation, we have observed a strong increase in pancreatic stone protein (PSP/reg) and pancreatitis-associated protein (PAP) in acinar cells. These proteins form a family of secretory stress proteins (SSP) that share sequence homologies (Unno et al. 1993; Graf et al. 2001). PSP/reg is constitutively expressed and secreted into pancreatic juice at micromolar levels (Bimmler et al. 1999a), whereas basal secretion of PAP is observed at nanomolar levels under physiological conditions. After a variety of insults, the expression and secretion of both proteins are highly increased (Rouquier et al. 1991; Graf et al. 2002). In vitro experiments with recombinant SSP or with pure pancreatic juice have demonstrated that exposure to trypsin results in the formation of insoluble fibrillar structures (Graf et al. 2001).

To characterize the expression profiles of the SSP in the WBN/Kob rat, we have measured PSP/reg and PAP levels in pancreatic juice and tissue at various time points during the development of the disease. Concomitant with the inflammation of acinar tissue and developing fibrosis, PSP/reg and PAP expression and secretion are markedly increased (Bimmler et al. 1999b). An initial increase in SSP immunoreactivity occurs parallel to changes in the architecture of individual acinar cells. A complete loss of reactivity is observed in areas with tubular complexes. In this paper, we demonstrate the re-organization of the secretory apparatus as demonstrated by SSP immunohistochemistry. Since the SSP are highly susceptible to tryptic cleavage with the consequences of fibril formation (Graf et al. 2001), the presence of intra- and extracellular fibrils strongly suggests that zymogens have been activated.

Materials and methods

Animals

All rats, purchased from BRL Füllinsdorf, Switzerland (64 male Wistar rats) and Japan LSC, Shizuoka, Japan (85 male WBN/Kob rats), were free of specific pathogens. Rats were housed in groups of no more than four in standard cages and kept in our animal facility for various periods between 1 week and approximately 6 months. Rats had free access to standard rat chow and water; they were maintained under specific-pathogen-free conditions at 20°C, with artificial lighting of 50 lux from 7 a.m. to 7 p.m., followed by almost complete darkness during the remaining hours. Relative humidity in animal rooms was 50%–60%.

Prior to sacrifice, the rats were deprived of food overnight (16–18 h) with free access to water. All manipulations conformed with Swiss federal guidelines on animal experiments and were approved by the local ethics committee.

Immunocytochemistry

To gain a representative sampling of pancreatic tissue, five pieces of 1–2 mm^3 were excised from each pancreas and embedded as described below. Pancreatic tissue specimens were fixed in freshly prepared 4% paraformaldehyde/0.1% glutaraldehyde (TAAB Lab., Aldermaston, UK) in phosphate-buffered saline (PBS) for 2 h at room temperature. Specimens were then washed extensively with PBS overnight at 4°C and embedded in plastic resin (Epon, Fluka, Buchs, Switzerland). Sections (1 μm thick) were mounted on SuperFrost-Plus-coated slides (Menzel-Gläser, Germany) and dried. The plastic was removed by immersion in a solution of 2 g KOH in 10 ml methanol/5 ml propyleneoxide for 7 min (Maxwell 1978). The solution was removed and the sections were rehydrated in a decreasing ethanol series. To unmask antigens, the sections were immersed in a solution of 0.01 M trisodium citrate, pH 6.4, and brought to the boiling point in a microwave oven (30 min). After being returned to room temperaure, the slides were rinsed in buffer and endogenous peroxidase activity was blocked by incubation in methanol containing 0.3% H_2O_2.

The Vectastain ABC-kit (Vector Laboratories, Burlingam, Calif.) was used for immunostaining according to the supplier's recommended protocol. Incubation was performed with primary antibodies, each being diluted 1:800 with 2% non-fat dry milk, and the sections were incubated for 2 h at room temperature with: (1) rabbit anti-rat PSP/reg (Bimmler et al. 1995), (2) guinea-pig anti-rat PAP I, (3) guinea-pig anti-rat PAP II, or (4) pre-immune serum from the animal that was used to raise the anti-rat PSP/reg. Guinea-pig antibodies raised against recombinant rat PAP I or PAP II were used as previously described (Schiesser et al. 2001; Graf et al. 2002). A solution containing 5 mM TRIS, pH 7.5, and 170 mM NaCl with 2% non-fat dry milk powder was used for all dilutions and washing steps.

Incubations with the biotinylated secondary antibodies and the ABC-reagent were performed as recommended and the sections were stained with a solution of 0.06% (w/v) DAB (3,3-diaminobenzidine tetrahydrochloride dihydrate, Fluka), diluted in 0.05 M TRIS, pH 7.5, containing 0.06% H_2O_2. All sections were processed in parallel and stained for 10 min. They were counterstained with haematoxylin, dehydrated through a graded ethanol series, cleared by xylene, and embedded in Histomount (National Diagnostics, Atlanta, Ga.).

Detection of PAP and PSP/reg coexpression was performed by confocal microscopy (Leitz, Germany). Epon sections were pretreated as above and blocked overnight at 4°C with 3% bovine serum albumin (BSA) dissolved in PBS. The sections were incubated with primary antibodies as above for 6 h at room temperature. After several washes in PBS, primary antibodies were detected with donkey anti-rabbit antibodies conjugated to Cy3 (Jackson Lab., Milan Analytica, La Roche, Switzerland) and rabbit anti-guinea-pig antibodies conjugated to fluorescein isothiocyanate (FITC; Sigma, Buchs, Switzerland), both diluted 1:100 in 3% BSA/PBS. Since the secondary antibody conjugated with FITC (rabbit anti-guinea-pig) might have been recognized by the other secondary antibody (donkey anti-rabbit), the secondary antibodies were applied sequentally: sections were first incubated in donkey anti-rabbit (Cy3) for 1 h, then washed in PBS, followed by incubation in rabbit anti-guinea-pig (FITC) for 1 h. The sections were washed again and embedded in Hydromount (National Diagnostics). Double-fluorescence detection on a Leitz confocal microscope was initially performed by the concurrent detection of the Cy3 and FITC channels. "Crosstalk", i.e. interference of the two signals was observed, and therefore sequential scans were run.

EM-immunogold technique

For the detection of antigens with the electron microscope, the same blocks as those used for light immunocytochemistry were trimmed and sectioned on an Reichert Ultramicrotome with a diamond knife (Diatome Ultra, Biel Switzerland). Sections were picked up on Formvar-treated nickel grids, blocked with 2% BSA in PBS containing 1.8% NaCl for 1 h and then exposed to 1:50 diluted primary antibody in the same solution for 2 h. The sections were extensively washed by repeated transfer into drops of fresh PBS and then immersed in blocking solution followed by Protein A-gold (EY Laboratories, San Matteo, Calif.) diluted 1:10 in PBS according to the manufacturer's recommendations. After 30 min, the grids were jet-washed by using a squirt-bottle filled with water and fixed in 1% glutaraldehyde. All manipulations were performed at room temperature. Counterstaining and contrast-enhancement were carried out by treatment with 5% uranyl acetate for 20 min. The sections were inspected on a Philips EM 208 microscope with a digital interface for direct viewing on a computer system.

Results

Co-expression of PAP and PSP/reg

In the pancreas of healthy Wistar rats, trypsinogen immunoreactivity was strong in all acinar cells and was localized predominantly to the zymogen granules (Fig. 1A). The cellular distribution of PSP/reg expression however varied considerably: most acinar cells exhibited a barely detectable level, whereas some single cells or cell clusters displayed high PSP/reg immunoreactivity

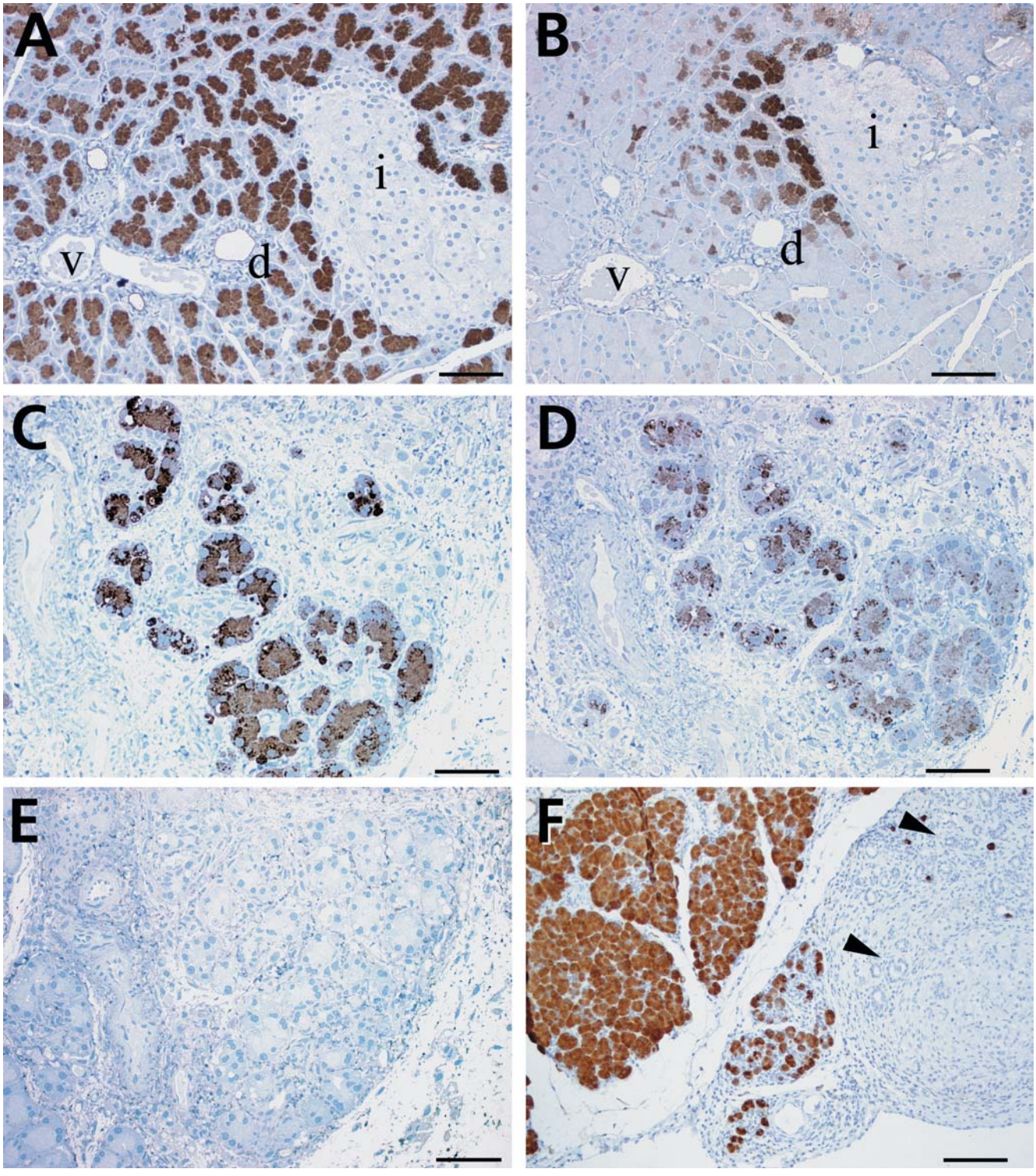

Fig. 1A–F Immunohistochemical evaluation of pancreas sections from WBN/Kob and Wistar rats. **A** Homogeneous acinar expression of trypsinogen in a 16-week-old Wistar rat (*i* islet, *d* duct, *v* vein). **B** Serial section of **A** stained with an antibody directed against PSP/reg. Single cells and groups of cells, often associated with islets (*i*) or ducts (*d*), are strongly positive, whereas the majority of acinar cells display only weak staining (*v* vein). **C** PSP/reg immunoreactivity in an area with strong inflammation and fibrosis in a 16-week-old WBN/Kob rat. **D** Serial section of **C** stained with an antibody directed against PAP II. **E** Serial section of **C** and **D** treated with a pre-immune serum. **F** Paraffin section of an intact lobule (*left*) and a lobule exhibiting changes of inflammation, fibrosis and formation of tubular complexes (*arrowheads*). *Bars* **A, B** 70 μm, **C–E** 30 μm, **F** 150 μm

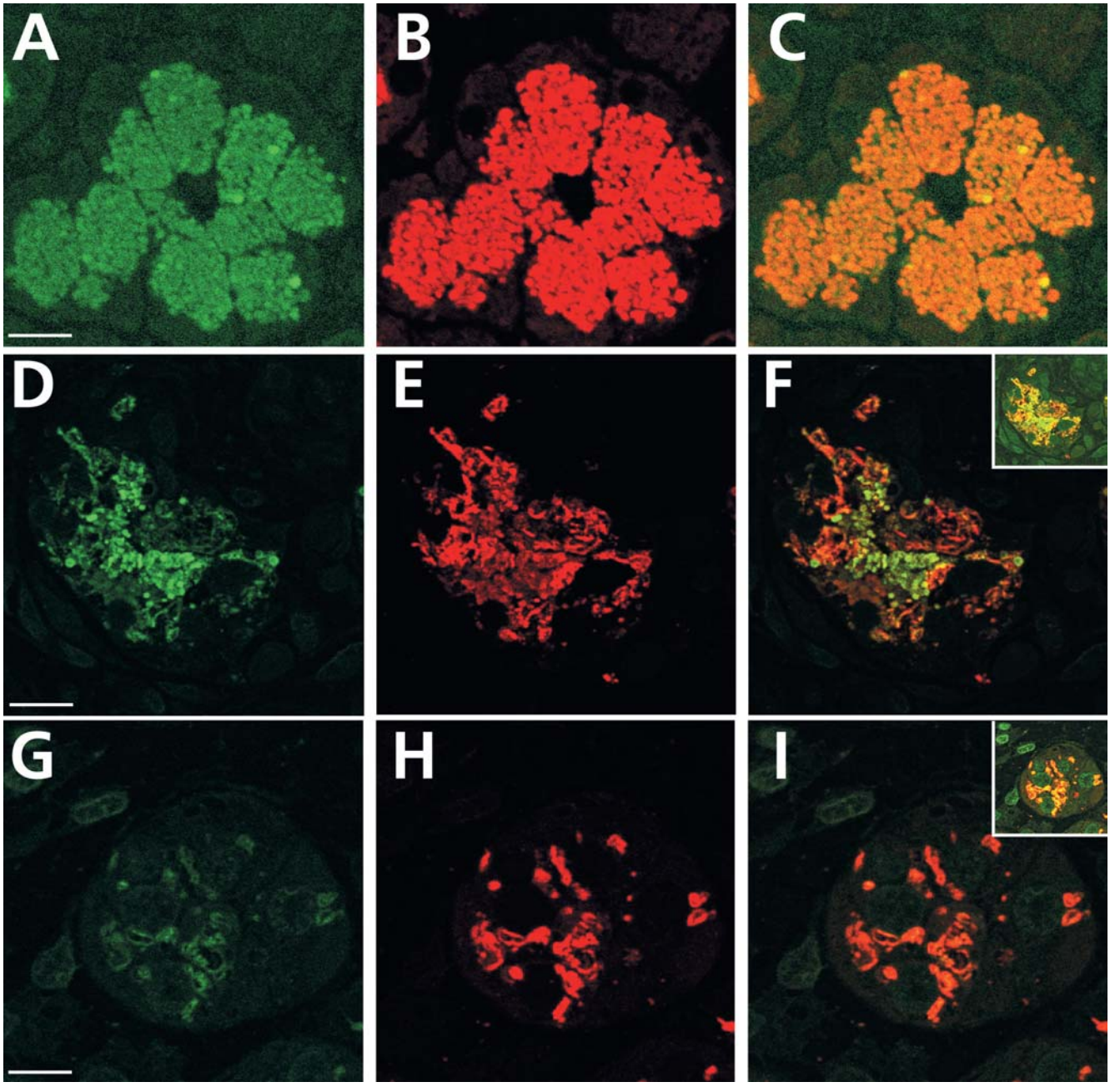

Fig. 2A–I Analysis of the secretory compartment by immunohistochemistry with antibodies against PSP/reg and PAP I. Plastic-embedded sections of pancreata from WBN/Kob rats were stained by double immunofluorescence: detection of PAP I (**A, D, G** *green*) and PSP/reg (**B, E, H** *red*) on the same section was achieved by confocal microscopy. **C, F, I** Electronic overlay (*yellow* coexpression, *green* PAP I, *red* PSP/reg) of the data from **A** and **B**, **D** and **E**, or **G** and **H**. *Inset* in **F**, **I**: Cellular shape and boundary of the acinus (overexposure) depicted in **D** and **E**, and **G** and **H**, respectively. *Bar* 8 μm

(Fig. 1B). The levels of PAP expression, though measurable in pancreatic secretions at nanomolar levels (data not shown), were not sufficient to be detected by immunocytochemistry.

In WBN/Kob rats, PSP/reg expression was markedly increased, particularly in inflamed areas and, at a lower level, in the surrounding tissue (Table 1). PAP I and PAP II immunoreactivity was detected in those cells that were strongly positive for PSP/reg. Serial sections of an area showing advanced destruction of acinar tissue exhibited PSP/reg (Fig. 1C) and PAP II (Fig. 1D) immunopositive material in similar patterns, suggesting that the different isoforms were expressed in the same cell. Application of a preimmune serum did not result in any visible staining (Fig. 1E).

Changes in acinar subcellular architecture were noted, particularly pertaining to the secretory apparatus. In

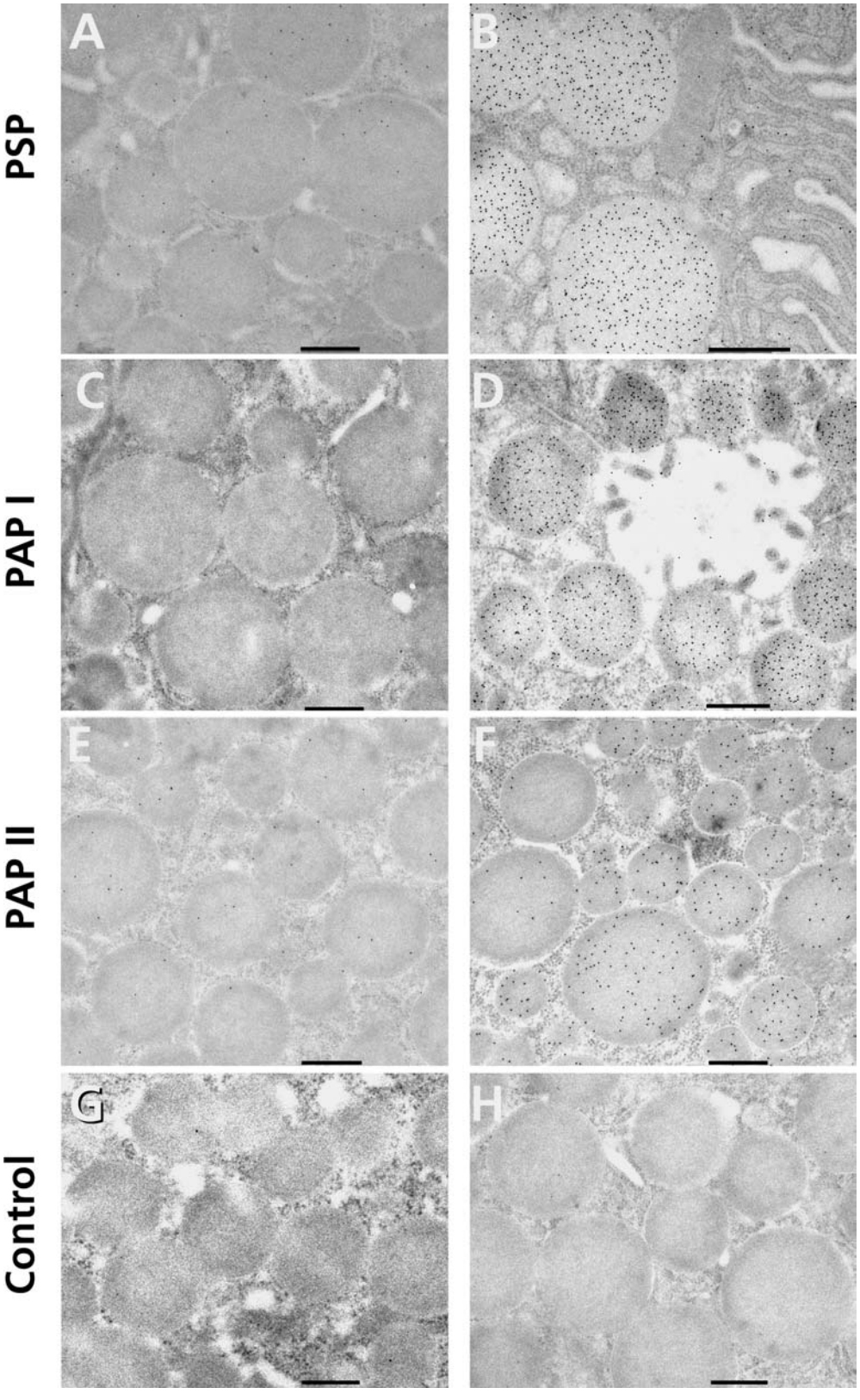

Fig. 3A–H Electron-microscopical (EM) analysis of secretory stress proteins in Wistar and WBN/Kob rats. EM sections were treated with antibodies directed against PSP/reg, PAP I and II. Antibodies were visualized with protein-A-bound gold particles. Wistar rats (**A, C, E**) and WBN/Kob rats (**B, D, F**): all 24 weeks old. **A, B** PSP/reg. **C, D** PAP I. **E, F** PAP II. **G, H** Controls: preimmune serum on sections of 24-week-old rats. **G** Wistar. **H** WBN/Kob. *Bars* 0.5 μm

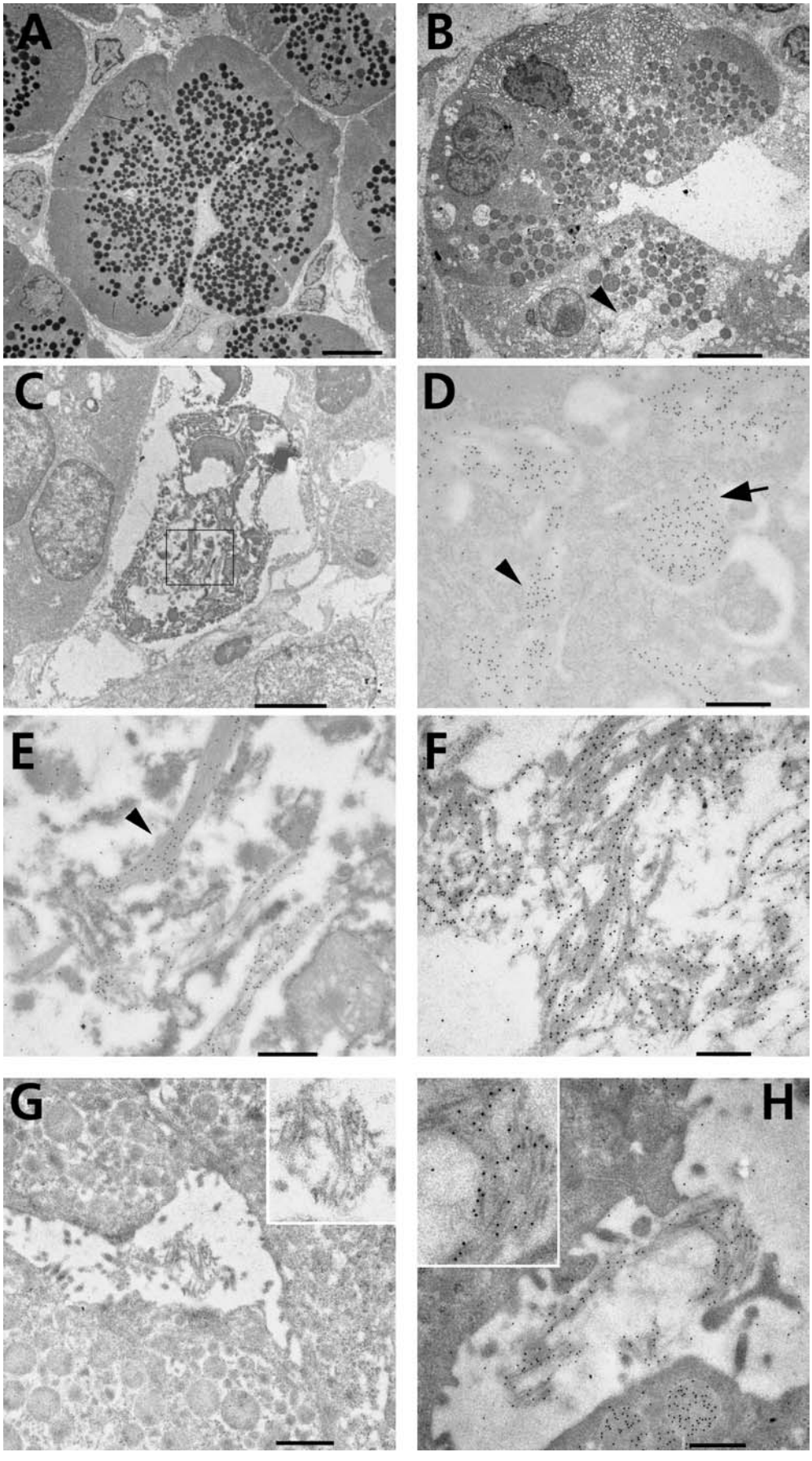

Fig. 4A–H Rearrangement of the secretory apparatus demonstrated by using immunogold detection of secretory stress proteins in the WBN/Kob rat. **A** Intact acinus of a Wistar rat. **B** Acinus of a WBN/Kob rat in the process of degeneration. Acinar cells exhibit oedema, nuclear condensation and large vacuoles (*arrowhead*). **C** Single acinar cell that has lost its normal cellular architecture. **D** Immunogold detection of PSP/reg. Intact and damaged secretory granules (*arrow* blebbing of zymogen granule, *arrowhead* elongated zymogen granule). **E** PSP/reg immunogold-positive fibrils; higher magnification of marked area in **C** (*arrowhead* first signs of fibril formation). **F** PAP I immunogold-positive fibrils in a cell that has totally lost its normal architecture. **G** Ductal lumen with fibrillar material. Control section with preimmune serum for immunogold detection. *Inset*: Detail of the fibrillar material showing no gold particles. **H** Immunogold visualization of PSP/reg-positive fibrils in a duct lumen. *Inset*: Detail showing gold-decorated fibrils. *Bars* **A** 9 μm, **B** 6 μm (original magnification: ×4,200), **C** 3 μm, **D** 0.4 μm (×54,000), **E** 0.5 μm, **F** 0.4 μm, **G** 0.9 μm, **H** 0.5 μm

Table 1 Summary of SSP-expression (*PSP/reg* and *PAP I*) in the pancreas of WBN/Kob rats after the onset of disease (– absence of any sign of SSP expression and absence of acinar damage or fibrosis, + <5% of acini SSP-positive with <5% acinar damage and <5% lobular fibrosis, ++ 5%–50%, +++ 50%–100%)

Week	Secretory stress protein		Acinar damage	Fibrosis
	PSP/reg	PAP I		
12	+	–	–	–
16	++	++	+	+
24	+++	++	++	++
36	++	+	+++	+++

addition, tubular complexes devoid of SSP immunoreactivity (Fig. 1F, arrows) were observed.

In order to characterize this process in more detail and to verify that the SSP colocalized to the same cells, we conducted an experiment with double-fluorescence labelling and confocal microscopy. The expression of SSP in the pancreas of WBN/Kob rats is characterized by an increase in immunoreactivity of PSP/reg and PAP I even in areas of minimal stress, i.e. in apparently unaffected tissue in the vicinity of an inflammatory region. An acinus from an unaffected area is shown in Fig. 2A–C. The polarized architecture of acinar cells is preserved with apically localized zymogen granules in which SSP are accumulated. Both PAP I (Fig. 2A) and PSP/reg (Fig. 2B) obviously colocalize to the same granules, as further demonstrated by the yellow-orange colour in the superposition (Fig. 2C).

Acinar cells within the inflammatory focus itself exhibited various degrees of rearrangement in the secretory apparatus. Figure 2D, F shows an acinus in an advanced stage of rearrangement. PSP/reg (Fig. 2D) and PAP I (Fig. 2E) were colocalized to the same cells. However, PSP/reg appeared concentrated in the basal region, whereas PAP I was apparently localized predominantly towards the apical pole. The overlay in Fig. 2F demonstrates this incomplete overlapping. In another advanced stage (Fig. 2G–I), the cells exhibited more condensed and apparently fused zymogen-granule-derived structues.

The first inflammatory changes in the pancreas of WBN/Kob rats were visible around the age of 12 weeks. In four out of five specimens at the age of 16 weeks and five out of five at the age of 24 weeks, multiple inflammatory foci were found that exhibited cellular rearrangements at the light-microscopical level, as shown above.

EM analysis of the secretory apparatus by using SSP-immunogold staining

To improve our understanding of the intracellular changes, we explored the acinar cell at the ultrastructural level by using the immunogold technique. We established the detection of PSP/reg, PAP I and PAP II immunoreactivity in WBN/Kob rats, which were expected to have a high content of SSP, and in Wistar rats with low SSP levels.

In Fig. 3, the immunogold staining of PSP/reg (Fig. 3A, B), PAP I (Fig. 3C, D) and PAP II (Fig. 3E, F) is demonstrated in the secretory compartment of 24-week-old Wistar and WBN/Kob rats, as visualized by electron microscopy. In WBN/Kob rats, which secrete high levels of SSP (Bimmler et al. 1999a), zymogen granules are positive for these antigens (Fig. 3B, D, F). Wistar rats secrete these proteins at much lower levels. Weak or no immunoreactivity is found in these aimals (Fig. 3A, C, E). Finally, preimmune serum used on sections from a Wistar (Fig. 3G) or a WBN/Kob (Fig. 3H) rat show a barely detectable non-specific reaction.

To explore the secretory apparatus in acinar cells in more detail, we selected specimens that exhibited inflammatory infiltration and fibrosis and that were positive for SSP immunoreactivity at the light-microscopical level. In addition, sections from healthy Wistar rats were inspected to ensure that changes were not atributable to preparation artifacts. Figure 4A shows an example of a healthy acinus. In contrast, an acinus with cells exhibiting various stages of cellular destruction or rearrangement is demonstrated in Fig. 4B. Vacuolization, enlarged lysosomal compartments and loss of the overall cellular structure are apparent. In more advanced stages (Fig. 4C), cellular compartments are completely destroyed and the cells appear “condensed”.

Within such cells, we found PSP/reg-immunoreactive material. Several stages of restructuring were identified. First, secretory granules lost their regular round appearance (arrow in Fig. 4D). The contents seemed to bleb in various directions but still had a smooth appearance (Fig. 4D). With the loss of defined cellular architecture, the granule contents formed elongated structures intermingled with endoplasmic reticulum (arrowheads in Fig. 4D, E). In the final stage, extensive fibrillar structures were visible in most of the cytosolic space. These strctures were immunoreactive for PSP/reg (Fig. 4D, E) and PAP I (Fig. 4F).

During the course of this study, we occasionally observed intraluminal fibrillar structures. Small ducts with electron-dense material of a fibrillar appearance were identified (Fig. 4G, H). We did not detect unspecific immunogold labelling of these fibrils by preimmune serum controls (Fig. 4G). However, distinct immunoreactivity was detected with antibodies against PSP/reg (Fig. 4H) and PAP I.

The presence of such intracellular and intraluminal fibrillar material suggests that trypsin had been prematurely activated.

Discussion

In this study, we have analyzed the structural changes of the apparatus for SSP in an animal model of chronic pancreatitis. The pathophysiology of chronic pancreatitis,

particularly with respect to the mechanism of acinar destruction, is not completely understood.

A characteristic feature of chronic pancreatitis is the occurrence of "tubular complexes" that resemble ductular structures. These tubular complexes have been interpreted as a consequence of ductal proliferation. Another theory states that they develop because of acinar-to-duct metaplasia. Convincing evidence supporting the second theory has recently been published (Bockman 1997; Wagner et al. 2001; Crawford et al. 2002). The formation of "tubular complexes" has also been observed in the WBN/Kob rat (Fig. 1F), indicating that early transformation of the cells and hence the loss of acinar function are similar to the changes seen in human pancreas.

When affected by inflammation and fibrosis, acinar cells of these animals may have two different fates: (1) apoptosis and removal by histiocytes or (2) a transdifferentiation process, viz. acinar-to-duct metaplasia. Both fates involve the removal of potentially dangerous zymogens.

We have shown that SSP are highly up-regulated in the vicinity of an inflammatory focus. Acinar cells within the inflammatory focus contain zymogen granules that fuse to form elongated organelles. Fibrillar structures are detectable within these elongated organelles and in the cytoplasm.

Since SSP are up-regulated under conditions of stress (Graf et al. 2002), SSP might have a protective function during the time of reorganization in the acinar cell. The appearance of intracellular PSP/reg-positive fibrillar structures is in accordance with our findings from previous in vitro experiments with recombinant SSP (Graf et al. 2001); in these experiments, we demonstrated that three out of four SSP have the capacity to form insoluble fibrils. These fibrils are generated after cleavage by trypsin. The presence of such fibrils is thus indicative of premature tryptic activity, which has been shown to be a key element of the pathophysiology in experimental (Luthen et al. 1995; Lerch and Gorelick 2000) and human hereditary (chronic) pancreatitis (Whitcomb et al. 1996).

The fibrils found in the WBN/Kob rat may contain proteins other than SSP, although for assembly, other components are not required as shown by in vitro experiments (Cerini et al. 1999; Graf et al. 2001).

The focus of this study has been to demonstrate the morphological changes in the secretory apparatus, with emphasis on the SSP. In the past, the presence of fibrillar material in the duct system (protein plugs) has gained much attention. We have now observed the rearrangement in the secretory apparatus with fibrils occurring in degrading zymogen granules.

Intracellular and intraluminal fibrils have been identified in a model of acute pancreatitis (retrograde injections of taurocholate). These fibrils have been shown to be immunoreactive for PAP by Morisset et al. (1997), although the authors were not sure whether the generation of fibrils was a direct result of bile infusion, even though they found fibrils in bile-treated animals only. Thus, fibril formation occurs in both acute and chronic pancreatitis. The observation of SSP-immunoreactive fibrils in the inflamed pancreas by two independent groups and the demonstration of the fibril-forming capacity of SSPs (Cerini et al. 1999; Graf et al. 2001) support the concept that the fibrils shown in this study consist of activated polymerized SSPs.

Acknowledgements We are grateful for the technical support and advice of the EM team headed by Prof. Thomas Bächi at the EM-Zentrum, Universität Zürich. The excellent technical help of R. Gassmann and M. Bain is highly appreciated. This report is a partial fulfillment of the requirements for the doctoral thesis of S.M.

References

Ashizawa N, Endoh H, Hidaka K, Watanabe M, Fukumoto S (1995) Scanning electron microscopic observation of pancreatic ducts in WBN/Kob rats. Pancreas 11:389–395

Bimmler D, Frick TW, Scheele GA (1995) High-level secretion of native rat pancreatic lithostathine in a baculovirus expression system. Pancreas 11:63–76

Bimmler D, Angst E, Valeri V, Bain M, Scheele GA, Frick TW, Graf R (1999a) Regulation of PSP/reg in rat pancreas: immediate and steady state adaptation to different diets. Pancreas 19:255–267

Bimmler D, Schiesser M, Angst E, Bain M, Frick TW, Graf R (1999b) In a rat model of chronic pancreatitis (WBN/Kob), levels of PSP/reg in pancreatic juice are highly increased in the early phase of the disease. Pancreas 19:416

Bockman DE (1997) Morphology of the exocrine pancreas related to pancreatitis. Microsc Res Tech 37:509–519

Cerini C, Peyrot V, Garnier C, Duplan L, Veesler S, Le-Caer JP, Bernard JP, Bouteille H, Michel R, Vazi A, Dupuy P, Michel B, Berland Y, Verdier JM (1999) Biophysical characterization of lithostathine. Evidences for a polymeric structure at physiological pH and a proteolysis mechanism leading to the formation of fibrils. J Biol Chem 274:22266–22274

Crawford HC, Scoggins CR, Washington MK, Matrisian LM, Leach SD (2002) Matrix metalloproteinase-7 is expressed by pancreatic cancer precursors and regulates acinar-to-ductal metaplasia in exocrine pancreas. J Clin Invest 109:1437–1444

Graf R, Schiesser M, Scheele GA, Marquardt K, Frick TW, Ammann R, Bimmler D (2001) A family of 16 kDa pancreatic secretory stress proteins form highly organized fibrillar structures upon tryptic activation. J Biol Chem 276:21028–21038

Graf R, Schiesser M, Lüssi A, Went P, Scheele GA, Bimmler D (2002) Coordinate regulation of secretory stress proteins (PSP/reg and PAP I, II and III) in the rat exocrine pancreas during experimental acute pancreatitis. J Surg Res 105:136–144

Hashimoto T, Yamada T, Yokoi T, Sano H, Ando H, Nakazawa T, Ohara H, Nomura T, Joh T, Itoh M (2000) Apoptosis of acinar cells is involved in chronic pancreatitis in WBN/Kob rats: role of glucocorticoids. Pancreas 21:296–304

Lerch MM, Gorelick FS (2000) Early trypsinogen activation in acute pancreatitis. Med Clin North Am 84:549–563

Luthen R, Niederau C, Grendell JH (1995) Intrapancreatic zymogen activation and levels of ATP and glutathione during caerulein pancreatitis in rats. Am J Physiol 66:66–66

Maxwell MH (1978) Two rapid and simple methods used for the removal of resins from 1.0 micron thick epoxy sections. J Microsc 112:253–255

Morisset J, Iovanna J, Grondin G (1997) Localization of rat pancreatitis-associated protein during bile salt-induced pancreatitis. Gastroenterology 112:543-550

Ohashi K, Kim JH, Hara H, Aso R, Akimoto T, Nakama K (1990) WBN/Kob rats. A new spontaneously occurring model of chronic pancreatitis. Int J Pancreatol 6:231–247

Rouquier S, Verdier JM, Iovanna J, Dagorn JC, Giorgi D (1991) Rat pancreatic stone protein messenger RNA. Abundant

expression in mature exocrine cells, regulation by food content, and sequence identity with the endocrine reg transcript. J Biol Chem 266:786–791

Schiesser M, Bimmler D, Frick TW, Graf R (2001) Conformational changes of pancreatitis-associated protein (PAP) activated by trypsin lead to insoluble protein aggregates. Pancreas 22:186–192

Unno M, Yonekura H, Nakagawara K, Watanabe T, Miyashita H, Moriizumi S, Okamoto H, Itoh T, Teraoka H (1993) Structure, chromosomal localization, and expression of mouse reg genes, reg I and reg II. A novel type of reg gene, reg II, exists in the mouse genome. J Biol Chem 268:15974–15982

Wagner M, Greten FR, Weber CK, Koschnick S, Mattfeldt T, Deppert W, Kern H, Adler G, Schmid RM (2001) A murine tumor progression model for pancreatic cancer recapitulating the genetic alterations of the human disease. Genes Dev 15:286–293

Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, Martin SP, Gates LK Jr, Amann ST, Toskes PP, Liddle R, McGrath K, Uomo G, Post JC, Ehrlich GD (1996) Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. Nat Genet 14:141–145