Pancreas transcription factor 1α expression is regulated in pancreatitis

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Abstract

**Background** Expression of acinar cell-specific genes requires the pancreas transcription factor 1α (Ptf1α). p48 is the only component of Ptf1α that is involved in both acinar gene regulation and pancreatic ontogenesis.

**Materials and methods** To determine whether Ptf1α/p48 expression is regulated during pancreatitis, acute pancreatitis was induced in rats by repeated caerulein injections; early chronic pancreatitis by the combined administration of caerulein and cyclosporin A; and focal pancreas fibrosis by trinitrobenzene sulfonic acid infusion into the pancreatic duct. AR42J cells were used to examine caerulein effects on acinar cells. Ptf1α/p48 expression was examined using immunohistochemistry, Western blotting, and qRT-PCR methods.

**Results** In acute pancreatitis, Ptf1α/p48 decreased markedly within 6 h as determined by Western blotting and immunohistochemistry. After 24 h, Ptf1α/p48 increased continuously and normalized at day six. In contrast, pancreas amylase reached a nadir at 48 h, when Ptf1α/p48 had largely recovered. In the early chronic pancreatitis model Ptf1α/p48 levels did not completely recover even at day 14, and this was associated with a failure to restore normal histology and amylase content. qRT-PCR showed that p48 mRNA were reduced after pancreatitis induction and were followed by a decrease in elastase mRNA. In the focal pancreas fibrosis model, Ptf1α/p48 expression was undetectable in areas with substantial acinar cell loss and tubular complexes. Caerulein did not affect Ptf1α/p48 expression in AR42J cells.

**Conclusions** Ptf1α/p48 protein and mRNA levels are regulated in acute and chronic experimental pancreatitis. Inability to re-express Ptf1α/p48 after injury may preclude acinar cell differentiation and appropriate pancreatic regeneration.

**Keywords** Chronic pancreatitis, differentiation, pancreas, pancreatitis, pancreatic regeneration, transcription factor.


X. Molero and T. Adell have made equivalent contributions to this manuscript and share first authorship.

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**Introduction**

Inflammatory diseases constitute the most common pathological conditions affecting the exocrine pancreas. Most frequently, acute pancreatitis is a self-limited disease that is associated with complete resolution of inflammation and no residual functional exocrine defect. Clinical and epidemiological evidence suggests that acute pancreatitis attacks often result in variable degrees of exocrine insufficiency that is transiently manifested days or weeks thereafter [1,2]. It is increasingly accepted that a severe attack or recurrent bouts of acute pancreatitis can lead to chronic pancreatitis [3,4]. A paradigm of this pathogenic sequence is chronic hereditary pancreatitis which is characterised by recurrent attacks of acute pancreatitis in young people or children [5]. Experimental evidence also indicates that multiple bouts of acute pancreatitis may lead to chronic inflammation and pancreatic atrophy [6]. In acute and chronic pancreatitis, the mechanisms governing exocrine pancreatic differentiation/proliferation are challenged. While no defect ensues in acute pancreatitis [1,2,4,7,8], loss of acinar cells and substitution by ductal complexes, accompanied by a marked desmoplastic reaction, take place in chronic pancreatitis [5,6]. Therefore, a better understanding of the mechanisms involved in the differentiation of exocrine pancreatic cells may provide clues as to the pathogenesis of these diseases. Because chronic pancreatitis constitutes a risk factor for the development of pancreatic cancer [9,10], such studies may also impact on a better understanding of the molecular mechanisms involved in this highly aggressive tumour [11].

The acinar differentiation programme depends on the activity of a pancreas transcription factor 1α (Ptf1α) complex constituted by basic helix-loop-helix (bHLH) proteins that bind the enhancer of acinar genes [12]: p75, p64, and p48 [13,14]. p75, encoded by E2A, is ubiquitous; by contrast, p48 is a tissue-specific bHLH protein that is expressed at high levels in the exocrine pancreas [15,16]. Recently, RBP-L (parologue of the mammalian Suppressor of Hairless – RBP-Jκappa) has been identified as a novel partner of the complex. RBP-L shares homology with RBP-Jκappa but displays a much more restricted tissue distribution [17]. There is much interest in understanding the role of tissue-specific transcription factors in the maintenance of the differentiated phenotype of acinar cells. An antisense construct targeting p48 blocked the expression of amylase and other acinar-specific genes in rat AR42J cells indicating that p48 is required for the activation of exocrine genes [16]. In addition, acinar-ductal transdifferentiation of normal human exocrine pancreatic cells and rat pancreatic tumour cells was associated with the selective down-regulation of p48 [15]. Nevertheless, the appropriate temporal and spatial expression of acinar genes requires additional regulatory proteins. Reintroduction of p48 by transfection into pancreatic cells displaying a ductal phenotype is not sufficient to activate the acinar phenotype [15,18, and unpublished data]. A complex containing Pdx1, Pbx1, and Meis that also binds to the promoter/enhancer of acinar genes has been shown to also play a fundamental role in their expression [19].

Additional interest in Ptf1α/p48 has arisen because this protein also plays a crucial role in the early development of the pancreas. Ptf1α/p48 is required not only for exocrine differentiation but also for normal islet development. Ptf1α/p48 knockout mice lack a pancreas and few cells expressing pancreatic hormones are present in the spleen [20]. Lineage tracing has shown that ventral pancreatic precursors that fail to express Ptf1α/p48 differentiate into intestinal cells [21]. Altogether, these studies have shown that for endodermal precursors to commit to pancreatic cells, both Pdx1 and Ptf1α/p48 are necessary [22]. Ptf1α/p48 has an antiproliferative effect mediated, in part, by the up-regulation of p21, suggesting that it couples cell proliferation and differentiation in the exocrine pancreas. The antiproliferative role of Ptf1α/p48 is substantiated by studies of cell proliferation in p48 −/− mice [23].

There is an increasing interest in elucidating the role that may play certain key transcription factors which govern the embryological cell differentiation programme in diseases of the adult pancreas. We have hypothesised that Ptf1α/p48 may be involved in the remodelling of the pancreas in inflammatory conditions and that some features of chronic pancreatitis, such as acinar atrophy and ductal complexes, might be associated with, and possibly due to, abnormal Ptf1α/p48 expression and consequent inability to activate acinar genes. Here we use several experimental strategies to induce pancreatitis in rats and examine changes in p48 expression. In addition, since normal acinar cells in culture undergo spontaneous ductal transdifferentiation [15,18], we use AR42J cells to examine possible direct effects of caerulein on Ptf1α/p48 expression. We show that acute oedematous pancreatitis is characterised by a self-limited down-regulation of Ptf1α/p48. By contrast, pancreatic injury leading to chronic pancreatitis is associated with a more persistent down-regulation of Ptf1α/p48. These findings support the notion that Ptf1α/p48 may be primarily involved in the pathogenesis of inflammatory diseases of the pancreas.

**Materials and methods**

**Induction of pancreatitis**

Acute oedematous pancreatitis was induced in Wistar male (approximately 300 g) fasted rats by administration of caerulein (Sigma Chemical Co, St. Louis, MO, USA) (20 µg kg−1) as two intraperitoneal injections given one hour apart, and animals were sacrificed at various time points after caerulein administration. To discriminate pancreatitis-specific effects, variable non-toxic doses of caerulein were administered to separate groups of rats. A minimum of 6 rats were included for each time point and dose of analysis. As a control, vehicle was administered.

Two protocols were used to induce chronic pancreatitis [24–26]. Cyclosporin A (CsA) administration together with caerulein results in impairment of pancreatic repair and...
morphological and biochemical modifications resembling early chronic pancreatitis [24,26]. Briefly, rats received one daily dose of CsA (Sandoz Pharma SAE, Barcelona, Spain) (20 mg kg⁻¹) given one hour apart. Animals were sacrificed and pancreata were collected and analysed for histology or biochemical assays at days six or 14 after caerulein administration. Administration of trinitrobenzene sulfonic acid (TNBS) into the pancreatic duct leads to established focal pancreatic fibrosis [25]. Briefly, Sprague-Dawley rats (300–350 g) were anaesthetized with ketamine (100 mg kg⁻¹ ip) and underwent cannulation of the biliopancreatic duct, which was tied close to the liver, and 0.4 mL of 2% TNBS in 10% ethanol/phosphate buffered saline (PBS) was infused retrogradely. Total exposure time to TNBS was 40 min followed by a washout of 30 min. Ligatures were released and the duodenum and abdominal wall sutured. Control rats were submitted to the same procedure except that PBS was administered. Rats were sacrificed 3 weeks later; tissue samples were processed as described below. Procedures were approved by the Animal Experimentation Committee of Hospital Vall d’Hebron.

**Tissue analysis of pancreatic enzyme activity**

Pancreas was collected and total weight, dry weight, and amylase content were determined. Pancreatic homogenates (100 mg mL⁻¹) were prepared by serial use of a motor-driven shearer (Tissue Tearer 985–370, Biospec Products Inc. Drewel, WI, USA) and Dounce homogenisers in cold buffer (50 mM sodium phosphate pH 7.3, 120 mM NaCl, 0.01% soybean trypsin inhibitor). Homogenates were sonicated prior to measurement of enzymatic activity in the supernatants. Amylase activity in total pancreatic homogenates was determined by the enzymatic colorimetric determination of pancreatic alpha-amylase (Sentinel CH, Milan, Italy) using an automated analyzer (Olympus AU5400, Olympus Optical Co. Ltd, Tokyo, Japan).

Results are provided as U/mg dry weight. To calculate dry weight, 200 mL of the initial homogenate was desiccated to dryness by heating at 160 °C for 16 h and then weighed.

**Western blotting**

Total pancreatic homogenates were prepared in liquid nitrogen in a mortar cooled in a bath of methanol with dry ice. The powder was transferred to a tube and proteins were solubilised in lysis buffer (3 mL buffer/piece of tissue of 0.5 x 0.5 cm). After vortexing, extracts were boiled for 15 min, centrifuged at 13 000 r.p.m. for 5 min at 4 °C, and the pellet was discarded. Protein concentration in the supernatant was determined using the DC Protein Assay (Bio-Rad, Hercules, CA, USA). Homogenates (100 mg) were fractionated by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and proteins were transferred to nitrocellulose filters by electroblotting. After blocking with 5% skim milk in Tris-buffered saline 1% Triton X-100 (TBST), filters were incubated with primary antibodies for 1 h, washed with TBST, and incubated with peroxidase-labelled goat antirabbit Ig. Reactions were revealed by enhanced chemiluminescence. Rabbit antiamylase antibodies were from Sigma. Affinity-purified rabbit antip48 antibodies were obtained as described elsewhere [15]. To normalize for protein loading, filters were incubated with antitubulin antibodies (1/2000).

**Immunohistochemistry**

Immunohistochemical assays were performed on fresh frozen tissue sections as described elsewhere [15]. Reactions were developed with diaminobenzidine. Sections were lightly counterstained with hematoxylin, dehydrated and mounted. Staining was performed using a Ventana automated system; all reagents were from Dako (Glostrup, Denmark). To detect amylase, rabbit polyclonal antibodies were used at 10 μg mL⁻¹. To detect keratin 19 (K19), a marker of ductal cells [27], LP2K hybridoma supernatant was used. Negative controls included pre-immune rabbit serum, irrelevant monoclonal antibody, or PBS.

**Quantitative RT-PCR analysis**

RNA was isolated from the pancreas of control rats as well as 2, 6, 24, 48 h and six days after caerulein treatment. Briefly, pancreata (six rats/group) were cut into pieces, placed in RNA Later (Qiagen, Valencia, CA, USA), and subsequently transferred to Trizol and homogenised; RNA was extracted following the manufacturer’s instructions. Samples with unacceptable RNA quality, estimated by very low RNA integrity number (RIN) using a bioanalyzer, were excluded. cDNA was synthesised using the High-Capacity cDNA Archive kit (Applied Biosystems, Foster City, CA, USA), and the equivalent of 50 ng RNA was used in each amplification reaction. Quantitative PCR was performed using TaqMan assays and ABI Prism 7900 Sequence Detection System equipment (Applied Biosystems, Foster City, CA, USA). Primers and probes for rat Ptf1α/p48, elastase, and hprt were designed by the Custom Assay Gene expression facility of Applied Biosystems. PCR parameters were 50 °C for 2 min, 95 °C for 10 min and 40 cycles of 15 s at 95 °C and 1 min at 60 °C. All reactions were done in triplicate. TaqMan software was used to calculate the threshold cycle (Ct) for each reaction, where the Ct value is the point in the extension phase of the PCR reaction at which the product is distinguishable from the background. Expression values for target genes were normalised to the concentration of hprt. Gene expression values were calculated based on the comparative Ct method [28], in which RNA control samples (basal) were designated as calibrators to which the other samples were compared. Experiments for the control samples were analysed together as one relative quantity (RQ). In short, the Ct data for all
genes under analysis and hprt in each sample were used to create delta-Ct (Ct target gene-Ct hprt). Thereafter, deltadeltaCt values were calculated by subtracting the deltaCt of the calibrator (basal controls) from the deltaCt value of each target. The RQs were calculated with the equation: \( RQ = 2^{-\text{deltadeltaCt}} \).

**Cell culture**

AR42J cells, derived from an azaserine-induced pancreatic tumour in rats, were obtained from N. Vaysse (Institut Louis Bougnard, Toulouse, France) and cultured in DMEM supplemented with 10% foetal bovine serum [15]. Cells were incubated with caerulein (0·05–500 nM) for 30 min, 1 h or 5 h. The effects of treatment with agonists specific for the cholecystokinin (CCK) receptor 1 (A71378, 0·13–130 nM) and 2 (SNF8702, 0·2–200 nM) (provided by D. Fourmy and M. Dufresne, INSERM U531, Toulouse, France), were assayed. Cells were collected and lysed in 25 mM Tris pH 7·5, 1 mM EGTA, 1 mM EDTA, 1% SDS, supplemented with a protease inhibitor cocktail. Lysates were boiled during 15 min, cleared by centrifugation, and protein levels were determined.

**Statistical analysis**

All numerical data are expressed as mean ± standard error of the mean. To compare the time-dependent effects of acute pancreatitis against basal conditions, one-way ANOVA with Dunnett’s test for multiple group comparisons versus control (basal group) was used.

**Results**

In normal adult pancreas tissue, Ptf1α/p48 is detected exclusively in the nucleus of acinar cells and is absent from ductal and islet cells (Fig. 1).

**Induction of acute oedematous pancreatitis with caerulein**

To induce a mild acute oedematous pancreatitis, caerulein (20 µg kg⁻¹ i.p.) was administered and rats were sacrificed after variable periods of time. At this dose, caerulein induces mild oedema and moderate inflammation. Six days after caerulein administration, pancreatic tissue appeared histologically normal, except for mild inflammatory cell infiltration, but most of the pancreatic tissue had recovered its normal architecture (Fig. 2). To examine Ptf1α/p48 expression, both Western blotting and immunohistochemical assays were used. By Western blotting, a marked decrease in Ptf1α/p48 expression was detected 6 h after caerulein administration which recovered partially at 24 h. There was some variation in the kinetics and extent of p48 decrease from experiment to experiment but the levels of Ptf1α/p48 were reproducibly close to normal 48 h after caerulein administration and had completely recovered by day six (Fig. 3). Similar results were obtained using immunohistochemical assays. In the normal pancreas, Ptf1α/p48 was detected exclusively in the nucleus of acinar cells. p48 expression was virtually undetectable by the 6 h time point. Nuclear staining of acinar cells with anti-p48 antibodies was again observed at 48 h (Fig. 2). Loss of Ptf1α/p48 immunoreactivity was unlikely to result from massive acinar cell damage given the minimal alterations in the histology of the pancreas and the lack of signs of cell death. To provide further support for this conclusion and provide better quantitative estimates of changes, pancreatic tissue amylase activity was also analysed. As shown in Fig. 3, activity was reduced by 31·2% at 24 h and by 51·2% at 48 h. By day six amylase activity had returned to almost normal values. Therefore, by the time the nadir of amylase activity and content was reached (48 h) Ptf1α/p48 protein levels had already normalised. Western blotting analysis also showed that loss of Ptf1α/p48 expression was not due to loss of amylase-containing acinar cells (Fig. 3). These results unequivocally indicate that the decrease in Ptf1α/p48 precedes acinar cell damage and does not result from a reduction in the content of acinar cells.

The same experimental protocol was applied using lower caerulein doses that do not induce acute pancreatitis (0·1–2 µg kg⁻¹ i.p.) and Ptf1α/p48 expression was analyzed using immunohistochemical assays. At these doses, loss of nuclear Ptf1α/p48 was not observed. At the 2 µg kg⁻¹ dose, a partial redistribution of Ptf1α/p48 to the cytoplasm of acinar cells was observed (data not shown).

To extend the findings reported above, qRT-PCR was performed using TaqMan assays. Results are shown in Fig. 4. Relative p48 mRNA levels showed a marked and significant decrease at the 2 and 6 h time points, remained low at the 24 h and were close to normal by 48 h. Transcript
levels at 6 d were similar to those of control rats. Changes in elastase mRNA levels were slightly delayed in comparison with p48 mRNA: levels were significantly lower than controls at 6 h, persisted abnormal at the 24 h and 48 h time points, and recovered by day six. Changes in p48 mRNA levels preceded changes in elastase mRNA levels. Overall, these findings are in agreement with the changes in protein levels observed.

Changes in Ptf1α/p48 expression in early stage chronic pancreatitis

The administration of CsA to rats for a total of 15 days, starting on day two, together with caerulein, is associated with a marked decrease in pancreatic weight, abundant inflammatory infiltrates, increased collagen content (Fig. 5), and frequent smooth muscle actin-expressing interstitial...
cells [24,26]. Using the experimental protocol described in the methods section, we could demonstrate that CsA treatment was associated with an impairment of Ptf1α/p48 recovery after caerulein pancreatitis. A marked decrease in Ptf1α/p48 levels was still observed at day six, as compared to the close to normal levels found in caerulein-induced pancreatitis without CsA treatment. This reduced Ptf1α/p48 expression persisted, though to a lesser extent, at day 14 (Fig. 5). Similar results were obtained using immunohistochemical assays. Pancreatic tissue amylase activity was also lower at days six (5·3 ± 1·9 U mg−1 dry weight) and 14 (3·3 ± 1·9 U mg−1) than in controls (25·6 ± 2·4 U mg−1) or caerulein-treated (19·6 ± 2·3 at day 6) rats (P < 0·001). These results indicate an association between recovery of Ptf1α/p48 and amylase levels upon pancreatic injury, suggesting that Ptf1α/p48 re-expression is required for the functional resolution of pancreatic injury.

Changes in Ptf1α/p48 expression in established TNBS-induced chronic pancreatitis

Unlike CsA + caerulein, TNBS induces a severe, focal form of chronic pancreatitis. Histologically, this lesion is associated with acinar atrophy, ductal complexes, infiltration by neutrophils and monocytes, and focal fibrosis three weeks after TNBS administration [25]. Immunohistochemical assays showed a marked decrease of Ptf1α/p48 and amylase expression in areas of chronic pancreatitis while preserved expression of both molecules was observed in areas of relatively normal pancreas. Amylase and Ptf1α/p48 were undetectable in K19-expressing cells in ductal complexes (Fig. 6).

Effects of caerulein and CCK receptor agonists on AR42J cells

The results presented above suggest that modulation of the activation of CCK receptor by caerulein might play a role in the regulation of Ptf1α/p48 expression. Despite attempts to reproduce the in vivo findings using rat acinar cell preparations, we did not observe major effects on Ptf1α/p48 protein levels (not shown). Normal acinar cell cultures were not used because they undergo a spontaneous ductal transdifferentiation associated with down-regulation of Ptf1α/p48 [15,18]. Therefore, we used AR42J cells and

Figure 3 Down-regulation of Ptf1α/p48 in acute caerulein-induced pancreatitis precedes down-regulation of amylase. (A) Western blotting analysis of Ptf1α/p48 (p48) and amylase content in pancreatic tissue (amyl: amylase). Analysis of the pancreas from two rats is shown at each time point. Ptf1α/p48 levels are down-regulated early on in the course of a mild pancreatitis induced by caerulein, but returns to normal in 48 h. The decrease in pancreatic amylase occurs subsequent to Ptf1α/p48 down-regulation. At 48 h, when Ptf1α/p48 levels are largely recovered, amylase expression is still greatly reduced. (B) kinetics of the quantitative analysis of pancreas amylase activity over the course of the experiment (n = 6). Results are expressed as mean±standard error of the mean; U/mg dry weight. (*P < 0·05. One-way ANOVA with Dunnett’s test for multiple group comparisons vs. basal).

Figure 4 Quantitative RT-PCR analysis of the levels of p48 and elastase mRNA after caerulein administration (comparative Ct method). Down regulation and recovery of Ptf1α/p48 expression precede changes in elastase expression. Results are expressed as mean±standard error of the mean (n = 6). * P < 0·05 vs. basal p48; # P < 0·05 vs. basal elastase. One-way ANOVA with Dunnett’s test for multiple group comparisons vs. basal.
Figure 5  Induction of early stage chronic pancreatitis by CsA + caerulein is associated with a decrease in Ptf1α/p48 levels in pancreatic tissue. (A) Microscopic findings in the pancreas two weeks after the first induction of pancreatitis with cyclosporin and caerulein. There is extensive loss of acinar cells and a marked increase in the amount of connective tissue. Scattered thin collagen fibres are seen in green colour, as shown by Masson-trichrome stain (Original magnification ×200). (B) Rats received CsA and caerulein as indicated in the Materials and Methods section and Ptf1α/p48 (upper layer) and amylase content (lower layer) were examined by Western blotting 6 and 14 days after caerulein administration. Samples from two different rats are shown at each time point. Unlike in rats receiving only caerulein, those receiving daily CsA display a defect in the recovery of Ptf1α/p48 levels that is associated with lower amylase tissue content. CR, caerulein; CCC, CsA + caerulein.

Figure 6  Induction of an established focal chronic pancreatitis by TNBS is associated with a marked decrease in Ptf1α/p48 and amylase expression in areas of ductal complexes. Histology (H-E, haematoxylin–cosin) and immunohistochemical analysis of the expression of Ptf1α/p48, amylase, and CK19. Nuclear Ptf1α/p48 and cytoplasmic amylase are detected in normal pancreas. By contrast, levels of Ptf1α/p48 and amylase are markedly decreased in chronic pancreatitis. Areas with well-preserved acinar tissue retain nuclear Ptf1α/p48 staining. The presence of abundant ductal complexes in TNBS-treated rats is evidenced by immunostaining for K19, a ductal marker. Sections incubated with antip48 antibodies were counterstained very lightly with haematoxylin in order to facilitate detection of low-level immunostaining. Asterisks indicate islets of Langerhans. Original magnification ×100, except for Ptf1α/p48 samples reproduced at ×200 to facilitate detailed inspection.
drugs acting on the various subtypes of CCK receptors. At the concentrations used (50 pm–500 nm), caerulein did not induce any changes in the levels of Ptf1α/p48 in AR42J cells after up to 5 h of treatment (Fig. 7). Furthermore, treatment of AR42J cells for 30 min with A71378 and SNF8702, CCKR1- and CCKR2-specific agonists, respectively, did not affect Ptf1α/p48 levels, as detected by Western blotting (Fig. 7).

Discussion

We have taken advantage of well-established models of experimental pancreatitis to show regulation of Ptf1α/p48 expression. Our findings suggest that this transcription factor plays an important role in the response to injury and functional regeneration of the pancreas. The findings are relevant since there is currently no information regarding the molecular mechanisms (i.e. cytokines, growth factors) involved in the regulation of Ptf1α/p48 expression.

Acute pancreatitis induced by supraphysiological doses of caerulein in the rat is characterized by diffuse inflammatory cell infiltration, oedema, acinar vacuolization and few necrotic cells. Full recovery is usually achieved within two weeks, making caerulein-induced acute pancreatitis a very useful model to study pancreatic repair. The time course of cellular and molecular events following caerulein challenge have been thoroughly studied [4,7]. Forty-eight hours after pancreatitis induction, fibroblast-like cells proliferate and total acinar mass is reduced. Signs of acinar cell recovery are evident five to seven days after injury, at a time when the number of fibroblasts and inflammatory cells is progressively reduced [26].

Here, we show that acute pancreatitis is associated with a very early down-regulation of Ptf1α/p48 in the pancreas, preceding changes in pancreatic amylase content and activity. These events were followed by a rapid and complete recovery of Ptf1α/p48, again preceding normalization of pancreatic amylase levels. Pharmacological modulation of caerulein pancreatitis with CsA leads to an impairment of pancreatic regeneration associated with a sustained reduction of Ptf1α/p48, suggesting that conditions that prevent Ptf1α/p48 re-expression or function may play a role in determining the fate of the injured tissue by promoting acinar cell mass reduction.

The dramatic down-regulation of Ptf1α/p48, the critical role of this protein in the expression of acinar genes, and its suboptimal recovery under conditions leading to early chronic pancreatitis strongly implicate Ptf1α/p48 in the pathogenesis of pancreatic insufficiency. Re-expression of Ptf1α/p48 seems required for acinar cells to reach a differentiated state, indicated by their ability to synthesise amylase. Given the straightforward hierarchical relationship between a transcription factor and its target gene(s), it is sound to propose that loss of Ptf1α/p48 plays a causal role in the loss of functional acinar cells. However, additional work is necessary to conclusively establish this relationship.

The TNBS model of established pancreatic fibrosis showed that Ptf1α/p48 is down-regulated in atrophic areas as well as in ductal complexes. The focality of the lesions in this model of pancreatitis provides an important control, showing that Ptf1α/p48 expression is maintained in areas displaying acinar phenotype and expressing amylase.

Our study does not address the mechanisms responsible for the down-regulation of p48. Supraphysiological doses of caerulein induce a sustained increase in (Ca++)i activation of MAPK and JNK pathways, and the activity of the NF-xB/Rel transcription factor through the sequential degradation of cytosolic IκB [29–31]. Physiological doses of caerulein are unable to activate NF-xB [31] and were unable to down-regulate Ptf1α/p48 expression (not shown), suggesting that pathways leading to NF-xB activation, or NF-xB itself, may be involved in Ptf1α/p48 degradation.

These observations led us to propose that caerulein-activated CCK receptors might participate in the down-regulation of Ptf1α/p48. Indeed, altered expression and cellular distribution of Ptf1α/p48 in transgenic mice expressing CCK2 receptors under the control of the elastase promoter has been demonstrated [32]. Our work using AR42J cells does not support such a notion, though several caveats should be considered: AR42J are neoplastic, constitutively release amylase, and express functional CCK1 and CCK2 receptors [33,34], unlike normal rodent acini which express exclusively CCK1 [35,36]. An alternative hypothesis is that the mechanisms operating in normal pancreas are inactive in AR42J cells.

Regarding the lack of recovery of Ptf1α/p48 in rats treated with caerulein + CsA, a role for mesenchymal cells can be proposed. CsA is able to increase the number of alpha-smooth muscle-expressing cells in the pancreas in the absence of effects on exocrine function [24,26]. However, it is possible that, upon acute acinar injury, normal...
regeneration is perturbed, leading to exocrine insufficiency. More work is necessary to clarify the relevance of these mechanisms to pancreatic insufficiency.

Altogether, the evidence supports the notion that down-regulation of Ptf1α/p48 participates in the functional loss of acinar characteristics that is typical of ductal complexes [37–39], as has previously been proposed using in vitro cellular models. Normal acinar cells undergo transdifferentiation to ductal cells upon in vitro culture: a loss of acinar marker expression and the acquisition of ductal markers, together with functional changes, takes place [40]. The mechanisms leading to loss of Ptf1α/p48 expression in vitro and in vivo need to be clarified. Recent work has led to the proposal that activation of the Notch pathway is an early event in the recovery from acute pancreatitis [41], in human chronic pancreatitis and in the development of ductal complexes in MT-TGF-α transgenic mice [42]. However, changes in p48 expression have not been thoroughly investigated, despite this being one of the most important transcriptional regulators involved in the activation of the terminal acinar differentiation programme.

Alternatively, induction of Ids (inhibitors of differentiation) might be involved. Ids are overexpressed in chronic pancreatitis and pancreatic cancer [43], suggesting a role for these inhibitors of differentiation. An additional mechanism is the mislocalization of Ptf1α/p48 to the cytoplasm. We have observed a predominant cytoplasmic distribution of Ptf1α/p48 in transfected pancreatic cancer cells displaying a ductal phenotype, in tissue samples from 1/4 human acinar cell carcinomas, in the acini of rats treated with low doses of caerulein and in some cases of established chronic pancreatitis [15], and data not shown.

Transcription factors involved in the activation of cellular differentiation play an important role in development as well as in the maintenance of normal tissue homeostasis and in recovery from injury. Conditional inactivation of Pdx-1, a major regulator of insulin gene expression, in adult mice leads to diabetes [44] and mice heterozygous for a Pdx-1 null allele display haploinsufficiency characterised by reduced glucose tolerance and an impairment in insulin secretion in old mice [45]. These effects are mainly due to an increase in islet cell apoptosis, indicating that regulators of tissue-specific genes can also modulate cell proliferation/apoptosis. Mice heterozygous for Ptf1α/p48 have been reported to be normal [20,21]. Our data nicely complement the findings made by Jensen et al. [41] and further point to a need for the recovery of the normal transcriptional programme for maintenance of exocrine function when subacute-chronic damage to the exocrine pancreas is induced. Gomez et al. [46] have also examined p48 mRNA levels in acute caerulein-induced pancreatitis in mice and found an up-regulation during the first 24 h after the last caerulein dose. Though these findings are in contrast to ours, it should be pointed out that rat and mice differ considerably in their response to caerulein [46,47]. Furthermore, these investigators quantitated p48 mRNA levels taking chymotrypsin mRNA as reference as they did not find changes in the levels of chymotrypsin after caerulein-administration, a finding that is in marked contrast with that of Jensen et al. regarding amylase mRNA levels [41], and with our own data presented here regarding elastase mRNA. Differences in mouse strain, pancreatitis induction protocol, and time frame of analysis may contribute to these variable observations. Additional work may be necessary to clarify these issues.

Altogether, these studies raise the concept that the analysis of genes coding for transcription factors involved in pancreatic differentiation/development in the adult pancreas may be valuable to clarify genetic susceptibility to exocrine failure. It is worth mentioning that Pdx1 mutations have been associated with pancreatic agenesis [48], mature onset diabetes of the young [49], and late onset type II diabetes [50,51], and Ptf1α/p48 mutations have been associated with neonatal diabetes and cerebellar agenesis [52]. It is, thus, conceivable that genetic variation in Ptf1α/p48 might be associated with chronic pancreatic insufficiency and/or diabetes.

In conclusion, our study shows that Ptf1α/p48 protein and mRNA levels are finely regulated in acute and chronic experimental pancreatitis. Inability to re-express Ptf1α/p48 after injury may preclude acinar cell differentiation and appropriate pancreatic regeneration. Thus, inappropriate Ptf1α/p48 expression may play important roles both in inherited and in acquired functional pancreatic insufficiency.

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