Regulation of PC4/TIS7 expression in adapting remnant intestine after resection

DEBORAH C. RUBIN, ELZBIETA A. SWIETLICKI, JOSEPH L. WANG, AND MARC S. LEVIN
Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110

Rubin, Deborah C., Elzbieta A. Swietlicki, Joseph L. Wang, and Marc S. Levin. Regulation of PC4/TIS7 expression in adapting remnant intestine after resection. Am. J. Physiol. 275 (Gastrointest. Liver Physiol. 38): G506–G513, 1998.—The adaptive response of the small intestine to loss of functional surface area includes enhanced crypt cell proliferation and enterocyte differentiation. To better define the underlying molecular and cellular mechanisms, we have cloned rat genes that are specifically regulated in the adaptive gut after 70% small intestinal resection. One of these is the immediate early gene PC4/TIS7. Compared with sham-resected control ileum, PC4/TIS7 mRNA levels in the adaptive remnant ileum were markedly increased at 16 and 48 h but not 1 wk after resection. Greater augmentation of PC4/TIS7 mRNA levels occurred in the ileum compared with the duodenum and proximal jejunum. After resection, the changes in intestinal PC4/TIS7 mRNA levels also exceeded changes in extraintestinal levels. The demonstration by in situ hybridization that villus-associated, but not crypt, cells express PC4/TIS7 mRNA is consistent with a role in regulating cytodifferentiation. The pattern of expression in the Caco-2 cell line is also consistent with such a role. Although the precise function of PC4/TIS7 in adaptation remains unclear, the early and intestine-specific changes in mRNA levels after 70% resection suggest that it might augment the adaptive response by stimulating the production of differentiated enterocytes.

Caco-2 cells; IEC-17 cells; IEC-18 cells; intestinal adaptation; enterocyte

THE MAMMALIAN small intestine contains a constantly proliferating and differentiating epithelium. The principal anatomic and functional unit of the small bowel epithelium is the crypt-villus axis. Stem cells in the crypts of Lieberkühn produce proliferating daughter cells that differentiate into the four major epithelial cell types during migration onto the villus or to the crypt base (2). Due to its ability to continuously proliferate and differentiate, this epithelium has a remarkable capacity to adapt to the loss of functional small bowel surface area. After surgical resection for the treatment of Crohn’s disease, ischemic injury, radiation enteritis, or other small bowel mucosal diseases, the residual intestine undergoes an adaptive response, resulting in an increased rate of crypt cell production and enhanced villus length and crypt depth. This ultimately leads to augmented absorptive capacity in the remnant adaptive gut (see Ref. 24 for review). Previous studies have shown that luminal factors, hormones, growth factors, and neural and vascular substances may be involved in initiating the adaptive response (24), although the underlying cellular and molecular mechanisms have not been elucidated. By clarifying the molecular basis of the adaptive response, specific therapies can be designed to enhance this process in patients with short bowel syndrome who are dependent on total parenteral nutrition.

We have been using a rat resection model of intestinal adaptation to address these issues. We have shown that there is a specific enterocytic component to the adaptive response. For example, the enterocyte responds to loss of small bowel surface area by increasing the expression of several enterocytic genes in the adaptive remnant ileum (23). Using subtractive hybridization techniques, we have cloned cDNAs that are specifically regulated in the adaptive remnant ileum postresection (4). Some of the gene products of these cDNAs are involved in regulating the cell cycle, protein synthesis and degradation, the response of the epithelium to acute injury, and nutrient uptake and trafficking (4).

This study describes the cloning and analysis of an immediate early gene, PC4/TIS7, that was isolated in an attempt to identify low-abundance cDNAs that might be important for initiating and maintaining the adaptive response. PC4/TIS7 is a member of the tetradecanoylphorbol ester-induced sequence (TIS) family of fibroblast genes induced by serum or 12-O-tetradecanoylphorbol-13-acetate (18, 27). This gene was originally identified because its expression is markedly increased early during nerve growth factor (NGF)-mediated cytodifferentiation of pheochromocytoma (PC)-12 cells into neurons (26). Data from nerve cell, muscle cell, and hematopoietic cell culture systems suggest that PC4/TIS7 plays a role in the cessation of cell division and the onset of cytodifferentiation (7, 8, 14, 16, 26). There have been few in vivo studies of PC4/TIS7 expression, and none addressing intestinal expression. Based on these observations and our demonstration that PC4/TIS7 mRNA levels are markedly increased in the adaptive remnant ileum early after resection, we have performed a detailed analysis of PC4/TIS7 expression in the adaptive gut. These studies of the cell-specific and temporal regulation of PC4/TIS7 and data from other systems suggest that PC4/TIS7 may be required for the onset of cellular differentiation. Although its role in adaptation remains unclear, PC4/TIS7 expression may be enhanced in adaptation in response to the requirement for increased numbers of differentiated enterocytes in the remnant ileum after massive intestinal resection.

MATERIALS AND METHODS

Animals. For intestinal resection and control surgeries, male Sprague-Dawley rats (225–260 g; Sasco, Omaha, NE) were acclimated to our animal care facility for at least 72 h. Rats were housed under strict light-dark cycles with free access to water and Teklad 7001 4% standard laboratory rat

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chow (Harlan Teklad Laboratory, Madison, WI). For analysis of gene expression during fetal intestinal ontogeny, precisely timed pregnant female Sprague-Dawley rats were obtained. Gestation day 0 was defined as the time of appearance of a vaginal plug. On gestation days 14, 18, 20, and 21, pregnant females were anesthetized with pentobarbital sodium and methoxyflurane, and pups were delivered by cesarean section. At least two full litters were used for each time point. For studies of postnatal ontogeny, at least two litters of suckling pups were used. All pups were maintained with their dams until they were killed.

Surgical procedures. Small bowel resection and transection control surgeries were performed as previously described (4, 23). Briefly, male Sprague-Dawley rats were subjected to either 70% small intestinal resection or transection control surgery. For 70% small bowel resection, the bowel was divided 5 cm distal to the ligament of TREITZ and 15 cm proximal to the ileocecal valve; the remnant jejunum was anastomosed to the ileum after removal of the intervening intestine. In the transected group, the bowel was divided 5 cm distal to the ligament of TREITZ and then reanastomosed end to end. Gentamicin (4 mg in 6 ml normal saline ip) was administered at the time of abdominal closure. All animals were provided with 5% sucrose in water containing oxytetracycline (4.5 g/l) for 24 h after surgery and were then allowed ad libitum access to rat chow. Animals that were killed at 1 wk after surgery were pair fed as previously described (23).

Tissues. At various times after resection or transection (2, 4, 8, 16, or 48 h or 1 wk), rats were killed and tissues (whole wall preparations) were rapidly frozen for RNA analysis or fixed in 4% paraformaldehyde in PBS for in situ hybridization analysis. Tissues included segments of duodenum and proximal jejunum from the preanastomotic site, and three equal segments of ileum (proximal, mid-, and distal ileum) harvested from the 15 cm of ileum proximal to the ileocecal valve. Equivalent segments were removed from the control transected animals. For ontogenic analyses, mid-small intestine (jejunum) was harvested from fetal day 18, 20, and 21 pups, and whole intestine was harvested from gestation day 14 pups. For the postnatal time points, jejunal and ileal segments were removed (n = 7–14 pups per time point from at least 2 litters). The number of false-positive clones.

Cloning of the PC4/TIS7 cDNA. A Lambda Zap II (Stratagene, La Jolla, CA) cDNA library was constructed using poly(A)1-enriched ileal RNA from 10 rats killed at 48 h, after 70% small intestinal resection (4). To obtain cDNAs that were transcriptionally regulated during adaptation, the library was screened with a high specific activity, subtracted cDNA probe as previously described (4). The experimental cDNA probe was generated by reverse transcription of the ileal RNA used for cDNA library construction. To enrich for genes specifically upregulated postresection, this cDNA was hybridized to a 15-fold excess of biotinylated poly(A)1 ileal RNA obtained from transected control rats, and RNA-cDNA hybrids were bound to streptavidin and extracted. After a second subtraction, the enriched cDNA probe was used to screen the cDNA library. The library was also screened with a control cDNA probe prepared analogously, starting with ileal RNA from transected rats that was subtracted with biotinylated ileal RNA from the resected rats.

To screen the adaptive ileal cDNA library, plaque lifts were done in duplicate and filters were prehybridized in 6× SSPE (1× SSPE is 0.15 M NaCl, 0.01 M Na2HPO4, and 0.001 M EDTA, pH 7.4), 5% SDS, 5× Denhardt’s solution, 50% formamide, and 50 µg/ml denatured salmon sperm DNA (4). The filters were hybridized to the experimental cDNA in this solution for 48 h at 42°C. Duplicate filters were sequentially screened with the experimental and control probes to reduce the number of false-positive clones.

DNA sequencing and analysis. cDNA clones were sequenced as previously described (4) by the chain-termination method using Sequenase 2.0 T7 DNA polymerase. Sequence data were analyzed with Genetics Computer Group Sequence Analysis software package version 7.1. GenBank and European Molecular Biology Laboratory databases were searched on the National Center for Biotechnology Information server, using BLASTn and TBLASTn algorithms (1).

RNA isolation and hybridization. Total RNA was isolated from intestinal segments (3). Northern blots (4, 23) were used to quantitate PC4/TIS7 mRNA levels in intestine during intestinal adaptation, during ontogeny in fetal and postnatal intestine, and in IEC-17, IEC-18, and Caco-2 cells. After hybridization with a high specific activity PC4/TIS7 cDNA probe, membranes were washed in 0.1× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) at 55°C, and autoradiograms were prepared. Kodak X-OMAT-AR (Eastman Kodak, Rochester, NY) films with Dupont Lightning Plus screens (Du Pont-NEN Life Science Products, Boston, MA) were exposed at −70°C for 21 days. Kodak BioMax MS films with the BioMax MS intensifying screen were exposed for 3 days. The relative abundance of each mRNA per sample was determined by NIH Image 1.55 (W. Rasband, National Institute of Mental Health) analysis of digitized images obtained with a UMAX PS-2400× scanner using UMAX Magiscan v. 1.2 (UMAX Technologies, Fremont, CA). Results were normalized for differences in RNA loading by digitized image analysis of 18S rRNA bands as visualized by ethidium bromide staining of RNA gels.

The temporal expression of PC4/TIS7 in adaptive and control intestine was evaluated by dot-blot hybridization of total RNA isolated from pooled ileal segments (n = 3 animals per time point). Only RNAs confirmed to be intact by denaturing gel electrophoresis were used. Washing and hybridization conditions were identical to those established for Northern blot hybridization, which identified a specific band for PC4/ TIS7 mRNA. For dot-blot hybridization studies, the relative abundance of PC4/TIS7 mRNA was determined by scanning laser densitometry (17).

In situ hybridization analysis. In situ hybridization analyses were performed as previously described (10, 22). Briefly, cryostat sections (6–8 µm thick) of tissues fixed in 4% paraformaldehyde in PBS were hybridized overnight at 42°C to an antisense or sense (control) PC4/TIS7 RNA probe.

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tion, sections were washed at 55°C in 0.1× SSC. Slides were dipped in NTB-2 photographic emulsion and developed in a Kodak D19 developer after 3–7 days. Multiple sections of ileum from at least three experimental (resected) and three control (sham resected) animals harvested 16 or 48 h postoperatively were subjected to in situ hybridization analysis. Sections were examined under dark-field microscopy, and photomicrographs were taken with a Nikon Microphot microscope.

Statistical analyses. Relative PC4/TIS7 mRNA levels in adaptive and transected control ileum that had been harvested at 8 and 48 h postoperatively and in Caco-2 and IEC-18 cell lines (proliferative vs. confluent and EGF treated) were compared with Student’s t-test and one-way ANOVA, using Excel 5.0 (Microsoft, Redmond, WA) statistical analysis software. Statistical significance was accepted at the P ≤ 0.05 level.

RESULTS

PC4/TIS7 is expressed in intestine, and mRNA levels are markedly increased in adaptive compared with control ileum. In previous studies, we demonstrated the feasibility of using subtractive hybridization techniques to clone cDNAs that are specifically upregulated in the adaptive ileum after massive small bowel resection (4). In the current study, prolonged autoradiographic exposures were used to help identify less-abundant cDNAs (i.e., more likely to represent transcription factors/immediate early genes important in initiating and maintaining the adaptive response). Using this approach, we isolated a 1.8-kb cDNA that encodes the rat PC4 immediate early gene (26), which is 97% homologous with the murine cDNA, TIS7 (18, 27). These genes were first isolated in an attempt to identify cDNAs induced in response to NGF-mediated PC-12 cell differentiation (PC4 (26)) or after mitogen treatment of 3T3 fibroblasts (TIS7 (18)).

To assess the role of PC4/TIS7 in adaptation, we first verified that its expression is increased in the remnant ileum after resection. Rats were subjected to 70% intestinal resection or transection control surgery. Adaptive and control ileal tissues were harvested at 8 and 48 h after resection for RNA isolation and Northern blot preparation. Northern blot hybridizations demonstrated that PC4/TIS7 mRNA levels were markedly increased in adaptive ileum (Figs. 1 and 2). Steady-state PC4/TIS7 mRNA levels were increased 5- to 10-fold in adaptive ileum compared with sham-resected controls (P < 0.02 at 8 h and P < 0.03 at 48 h).

The increase in PC4/TIS7 mRNA levels during adaptation is temporally and spatially regulated. The temporal course of the adaptive response after massive intestinal resection is complex. One of the first events is an increase in the rate of crypt cell production, which begins as early as 16 h postoperatively (29), followed by the establishment of villus hyperplasia and an enhanced rate of enterocyte migration (by 1–2 wk, maximal at 1 mo (5)). Thus we conducted a detailed analysis of the temporal regulation of PC4/TIS7 expression. Rats underwent 70% intestinal resection and tissues were harvested at 2, 4, 8, 16, 48, and 168 h (1 wk) after surgery (n = 3 per group; Fig. 3). PC4/TIS7 mRNA levels rose in adaptive gut by 8 h after resection. The maximal increase in PC4/TIS7 mRNA expression in adaptive compared with control sham-resected ileum occurred at 16 h postoperatively (Fig. 3A); however, relative mRNA levels were highest in adaptive ileum at 48 h after resection (Fig. 3B). PC4/TIS7 mRNA levels then decreased to baseline by 1 wk.

To determine whether PC4/TIS7 mRNA levels are also increased in other regions of the gut during adaptation or whether the increase is specific to the ileum, PC4/TIS7 mRNA levels were quantitated in the duodenal and jejunal segments at 48 h after resection or transection surgery. PC4/TIS7 mRNA levels were
significantly increased postresection in the duodenum (3.7-fold; \( P < 0.01 \)) but not in the jejunum.

Cell-specific expression of PC4/TIS7. The gut epithelium is complex, composed predominantly of absorptive enterocytes, goblet cells, enteroendocrine cells, and Paneth cells. In addition, the bowel wall contains myenteric neurons and circular and longitudinal muscle layers. To provide clues to the function of PC4/TIS7 in intestinal adaptation, in situ hybridization analyses were performed to determine the cell-specific expression of PC4/TIS7 in the intestine (Fig. 4). In adaptive ileum, PC4/TIS7 mRNA was present in villus-associated enterocytes located all along the villus (Fig. 4A), extending to the villus base, but not in the crypts (Fig. 4B). Ileal sections hybridized to a sense control probe did not demonstrate detectable signal (Fig. 4C).

PC4/TIS7 expression in fetal and postnatal rat intestine. Data from other laboratories suggested that PC4/TIS7 expression is required for the onset of neuronal and muscle cell differentiation (7, 8, 26). The rat intestinal epithelium arises from undifferentiated endoderm during the last week of the 21-day gestation period (fetal days 14–21), and cytodifferentiation of the gut epithelium continues through the first 4 wk of life. To further assess the role of PC4/TIS7 in the gut, its expression in the developing intestine was determined during the initiation of cytodifferentiation in fetal and postnatal life. Segments of intestine were harvested...
PC4/TIS7 mRNA expression in fetal intestines

PC4/TIS7 cDNA was originally cloned from neural PC-12 cells and from NIH/3T3 fibroblasts, and it is expressed in multiple extraintestinal tissues, including placenta, amnion, heart, spleen, and skeletal muscle. To determine the tissue specificity of the adaptive increase in PC4/TIS7 mRNA levels, Northern blot hybridization was performed. The lungs, liver, kidneys, heart, testis, and brain were removed from sham-operated control adult rats and from rats subjected to 70% intestinal resection and sham transection surgery, 16 and 48 h postoperatively. In normal unoperated controls, PC4/TIS7 mRNA was detectable at low levels in all of these tissues (data not shown). Although the increase in intestinal PC4/TIS7 mRNA levels was maximal in adaptive gut at 16 h after resection, there was no significant change in mRNA levels compared with transection controls in any extraintestinal tissue at 16 h after resection. At 48 h after resection, there was a 1.6- to 1.9-fold increase in PC4/TIS7 mRNA expression in the liver, kidneys, and lungs of resected rats compared with sham-resected animals (compared with a 4- to 5-fold increase in intestinal mRNA levels). mRNA levels were unaltered in brain, testis, and heart.

PC4/TIS7 mRNA expression in intestinal epithelial cell lines. In the adapting intestine, PC4/TIS7 mRNA is detectable in enterocytes located from the villus base to the villus tip but could not be found in crypt cells, as determined by in situ hybridization techniques. To identify an in vitro model to test the hypothesis that PC4/TIS7 expression may be increased in adaptive enterocytes in response to a requirement for accelerated production of differentiated cells, we examined PC4/TIS7 expression in Caco-2 cells and in IEC-17 and IEC-18 cells. After achieving confluence, the human colon cancer-derived Caco-2 cell line forms polarized monolayers that acquire many features of the small intestinal mucosa. PC4/TIS7 mRNA is readily detectable in Caco-2 cells by Northern blot hybridization. Although it was expressed at equivalent levels in cells 1 day preconfluence and 3 and 7 days postconfluence, the level of expression by 14 days postconfluence was 15-fold higher than at the earlier time points (Fig. 6A). IEC-17 and IEC-18 cells are rat cryptlike cells derived from rat duodenum and ileum, respectively. They retain an undifferentiated phenotype in culture but have the potential for differentiation into enterocytes and goblet cells (15, 25). Although PC4/TIS7 was abundantly expressed in proliferating IEC-17 and IEC-18 cells, mRNA levels were markedly decreased in confluent, quiescent cells (Fig. 6B and C).

EGF increases PC4/TIS7 mRNA levels in IEC-18 cells. EGF treatment of NIH/3T3 cells causes a rapid increase in PC4/TIS7 expression (18), and EGF rapidly stimulates the expression of several immediate early genes such as c-fos, c-jun, and jun b (13). We examined the effect of EGF exposure on PC4/TIS7 mRNA expression in IEC-18 cells. PC4/TIS7 mRNA levels significantly increased after exposure to EGF (250 ng/ml; Fig. 7). Thus the response of PC4/TIS7 to EGF exposure in intestinal epithelial cells is similar to that of NIH/3T3 fibroblasts.

DISCUSSION

Our previous analyses of gut adaptation led to the cloning of several classes of genes that are transcriptionally regulated in the first 48 h after resection (4, 23). Included were genes with important roles in cell cycle regulation, in protein synthesis and degradation, or in intestinal nutrient uptake and trafficking. In the present study, we describe the identification and characterization of a less abundantly expressed immediate early gene, PC4/TIS7, that is markedly upregulated early after small bowel resection.

PC4/TIS7 was originally cloned from PC-12 cells in an attempt to isolate cDNAs that were induced after stimulation by NGF to induce cytodifferentiation (26). Simultaneously, it was also identified as a member of the TIS family of fibroblast genes that are induced by growth factors during cell growth and differentiation (27). The PC4/TIS7 gene is expressed in multiple extraintestinal tissues, including placenta, amnion, heart, spleen, and skeletal muscle. To determine the tissue specificity of the adaptive increase in PC4/TIS7 mRNA levels, Northern blot hybridization was performed. The lungs, liver, kidneys, heart, testis, and brain were removed from sham-operated control adult rats and from rats subjected to 70% intestinal resection and sham transection surgery, 16 and 48 h postoperatively. In normal unoperated controls, PC4/TIS7 mRNA was detectable at low levels in all of these tissues (data not shown). Although the increase in intestinal PC4/TIS7 mRNA levels was maximal in adaptive gut at 16 h after resection, there was no significant change in mRNA levels compared with transection controls in any extraintestinal tissue at 16 h after resection. At 48 h after resection, there was a 1.6- to 1.9-fold increase in PC4/TIS7 mRNA expression in the liver, kidneys, and lungs of resected rats compared with sham-resected animals (compared with a 4- to 5-fold increase in intestinal mRNA levels). mRNA levels were unaltered in brain, testis, and heart.

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serum or by 12-O-tetradecanoylphorbol 13-acetate (18, 27). PC4/TIS7 expression is also increased in response to EGF and fibroblast growth factor (FGF) in normal fibroblasts and in those with suppressed protein kinase C (PKC) activity (18, 19). Thus PC4/TIS7 expression may be regulated by multiple ligands, in PKC-dependent and -independent pathways. No transcription factor motifs have been identified in the PC4 or TIS7 promoters. Sequence analysis demonstrates homology with murine interferon-β and rat interferon-γ, but there is no evidence that PC4/TIS7 has antiviral activity or is actively secreted.

We have shown that PC4/TIS7 mRNA levels are markedly increased during the first 48 h after 70% intestinal resection. By in situ hybridization analysis, PC4/TIS7 mRNA was found in adaptive villus-associated enterocytes located from the villus base to tip but was not present in crypt cells. Although PC4/TIS7 was expressed in many extraintestinal tissues, adaptive changes in its expression after resection were almost completely limited to the gut. In the liver, kidneys, and lungs, changes in expression were only observed at 48 h after resection, and these were modest compared with intestinal changes. As described below, PC4/TIS7 mRNA was also expressed in several intestinal epithelial cell lines including IEC-17, IEC-18, and Caco-2 cells. Although both preconfluent proliferating and postconfluent differentiating Caco-2 cells produce PC4/TIS7 mRNA, the level of expression was markedly increased at 14 days postconfluency, when the differentiated

![Fig. 7. Epidermal growth factor (EGF) induces PC4/TIS7 mRNA expression. IEC-18 cells were grown for 7 days (3 days postconfluence) as per MATERIALS AND METHODS. Cells were serum starved for 24 h and then treated with EGF (1, 10, or 250 ng/ml) for 30 min and harvested. Total RNA was electrophoresed and transferred to membranes, and Northern blot hybridization was performed using a radiolabeled PC4/TIS7 cDNA probe. Bands specific for PC4/TIS7 mRNA were quantitated as indicated in MATERIALS AND METHODS. Data are expressed means ± SE. *P < 0.05.](image-url)

![Fig. 6. Northern blot demonstration that PC4/TIS7 mRNA is expressed in intestinal Caco-2, IEC-17, and IEC-18 cell lines. RNA was isolated and Northern blots were prepared and hybridized with radiolabeled PC4/TIS7 cDNA as in Figs. 1 and 2. A: Caco-2 cells, grown as described in MATERIALS AND METHODS, were harvested at 1 day preconfluence and at 3, 7, and 14 days postconfluence (pc). Inset: typical autoradiogram (96-h exposure) of a Northern blot hybridized with radiolabeled PC4/TIS7 cDNA. Each lane was loaded with 10 µg of total cellular RNA prepared from an independent flask of cells. Bar graph summarizes the quantitative analysis. Relative abundance of each mRNA sample was determined as per MATERIALS AND METHODS. Data are means (n = 4) ± SE in arbitrary units after correcting for RNA loading with 18S rRNA. *P < 0.001 for ANOVA and for 14-day data compared directly with each of the other time points. B: IEC-17 (lanes 1–4) and IEC-18 cells (lanes 5, 6, 11, and 12) were harvested in log phase (even-numbered lanes) or at confluence (odd-numbered lanes). C: quantitation of Northern blot hybridization represented in B. Relative abundance of each mRNA sample was determined as per MATERIALS AND METHODS. Data are expressed as means ± SE for all log phase (proliferating) IEC-18 cells (IEC-18p), confluent (quiescent) IEC-18 cells (IEC-18q), log phase IEC-17 cells (IEC-17p), or confluent IEC-17 cells (IEC-17q). *P < 0.04.)
phenotype is fully expressed. Expression was downregulated in quiescent intestinal epithelial cells, but mRNA levels increased in these cells after exposure to EGF.

The role of PC4/TIS7 in the normal intestinal epithelium and after loss of functional small bowel surface area has not been previously investigated. Based on studies in neural and muscle cell lines, PC4/TIS7 is likely to have an important role in regulating the onset of cellular differentiation in the small intestine. When rat PC-12 pheochromocytoma cells are exposed to NGF, cell division is inhibited and differentiation into mature sympathetic neurons is stimulated (26). PC4/TIS7 mRNA and protein levels increase during the early differentiation phase, and PC4/TIS7 is translocated from the cytoplasm to the nucleus. This translocation is reversible on removal of NGF, paralleling the differentiation state of the cell (8). The nuclear translocation of PC4/TIS7 may be tissue specific, since similar changes were not found in fibroblasts (28).

The potential role of PC4/TIS7 in cytodifferentiation has also been explored in the murine C2C12 myoblast and the 32DC13(G) hematopoietic cell lines. In C2C12 cells, myotubes are formed by myoblast fusion, accompanied by a well-defined activation of a series of muscle-specific regulatory genes. Inhibition of PC4/TIS7 expression in these cells by antisense transfection led to markedly decreased expression of myogenin and myosin, and myotube formation was delayed (7). The hematopoietic progenitor cell line 32DC13(G) differentiates in response to granulocyte colony-stimulating factor (GCSF), which induces these cells to terminally differentiate into neutrophilic granulocytes. PC4/TIS7 mRNA levels increase in response to GCSF (16). Transfection of v-abl and v-ras interferes with GCSF-induced differentiation in association with a block in the normal increase in PC4/TIS7 mRNA levels. Release of the differentiation block in ras-transformed cells by retinoic acid and GCSF restored the PC4/TIS7 response. Thus most studies in a variety of cell types support the notion that PC4/TIS7 is required for the onset of cellular differentiation.

The earliest phase of adaptation after resection is characterized by enhanced crypt cell proliferation, leading to increased production of newly differentiating epithelial cells. Our studies provide indirect evidence that the role of PC4/TIS7 in intestinal adaptation may be similar to its postulated role in other systems, namely in initiating cellular differentiation after withdrawal of proliferating cells from the cell cycle. PC4/TIS7 expression is markedly increased as early as 8 h after small bowel resection, coinciding with the onset of enhanced crypt cell proliferation and accelerated production of newly differentiating villus cells. In situ hybridization analysis shows that PC4/TIS7 mRNA is readily detectable in nascent villus-associated cells as they exit from the crypts but is not present in vivo in proliferating cells. Finally, at early times after resection, its expression is most prominently increased in the adaptive ileum, the region of the intestine that demonstrates the most prominent morphological response to small bowel resection (5).

The reason for the selective increase in PC4/TIS7 expression in adaptive duodenum and ileum but not jejunum is not immediately obvious, since a jejunal morphological adaptive response has been described (24). We have observed that the expression of another enterocytic gene, apolipoprotein A-I-V, was increased in adaptive ileum and duodenum but not in jejunum at 48 h and 1 wk after resection (23). It is possible that jejunal enterocytes already express maximal levels of their repertoire of enterocytic genes and cannot respond to extracellular signals to further enhance gene expression. Analysis of expression patterns of other classes of enterocytic genes in adaptive jejunum and ileum should help further clarify the underlying mechanisms.

The temporal changes in expression of PC4/TIS7 are different from other immediate early genes of the TIS family. The expression of c-fos and c-jun has been studied in an adaptive fasting-refeeding model. mRNA levels for both genes were found to be transiently increased 2 h after refeeding (12). In contrast, in the resection model, PC4/TIS7 mRNA levels were unchanged at 2 and 4 h but increased by 8 h, remaining elevated at least as late as 48 h postoperatively. These data support the conclusion that PC4/TIS7 is different from other immediate early genes, which was based on its homology with interferons (26) and its pattern of expression during NGF-induced differentiation of PC-12 cells (8).

Data from the intestinal epithelial cell lines examined in this study indirectly support a role for PC4/TIS7 in the initiation of cytodifferentiation. As discussed above, PC4/TIS7 mRNA is expressed in 1-day preconfluent, proliferating Caco-2 cells, which have the potential for differentiation, and at much higher levels in fully differentiated 14-day postconfluent cells. On the other hand, proliferating intestinal epithelial cells express PC4/TIS7 mRNA, but levels decrease dramatically at confluence. Although these cells become quiescent when confluent, spontaneous differentiation does not occur in culture. These results suggest that expression of PC4/TIS7 is not sufficient to induce the onset of cytodifferentiation but may be necessary for differentiation to occur (possibly as a coactivator of other tissue-specific factors). Suh and Traber (25) have shown that transfection of intestinal epithelial cells with the Cdx-2 homeobox gene leads to enterocytic and goblet cell differentiation in culture. Analysis of the regulation of PC4/TIS7 expression in these cells could help elucidate its role in gut differentiation.

The increase in PC4/TIS7 mRNA levels in adaptive intestine after small bowel resection is not likely to be part of a generalized injury response. Expression does not increase at 2 and 4 h after surgery, when ischemic/hypoxic injury to the gut would be maximal. Also, the expression persists at relatively increased levels at least 48 h after resection. In addition, PC4/TIS7 expression is not increased in a hypoxic/ischemic brain injury model or in a facial nerve transection model of nerve injury (9, 11). The small increase in PC4/TIS7 expression in rat kidneys, lungs, and liver (and not heart or
brain) after 70% resection but not sham resection suggests that resection produces changes in circulating, epithelium-specific extracellular signals that act predominantly on the gut but can also affect other epithelial tissues.

The specific activation of the PC4/TIS7 gene during the early stages of the adaptive response will help to more selectively identify putative factors that may be involved in initiating this process. As noted above, PC4/TIS7 mRNA levels increase in response to a variety of growth factors including NGF, EGF, and basic FGF. Regulation by EGF is of particular interest in intestinal adaptation, since intravenous and oral administration of EGF increases cellular proliferation and enhances gut adaptation (6, 20). Enhanced mucosal mass and adaptation have been shown after administration of EGF to the rabbit (21). Further studies in intestinal epithelial cell lines may permit the identification of other growth factors that regulate PC4/TIS7 expression in the gut, which in turn will help to elucidate the complex regulatory pathways that underlie the adaptive response. This knowledge will facilitate the development of more specific therapies for the treatment of short bowel syndrome and for intestinal dysfunction resulting from diffuse mucosal diseases.

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