Transcriptional regulation of the lactase-phlorizin hydrolase promoter by PDX-1

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Wang, Zhi, Rixun Fang, Lynne C. Olds, and Eric Sibley. Transcriptional regulation of the lactase-phlorizin hydrolase promoter by PDX-1. Am J Physiol Gastrointest Liver Physiol 287: G555–G561, 2004. First published April 23, 2004; 10.1152/ajpgi.00011.2004.—Lactase-phlorizin hydrolase gene expression is spatially restricted along the anterior-posterior gut axis. Lactase gene transcription is maximal in the distal duodenum and jejunum in adult mammals and is barely detectable in the proximal duodenum. By contrast, pancreatic duodenal homeobox-1 (PDX-1) protein is expressed maximally in the proximal duodenum. This study aimed to determine the role of PDX-1 in regulating lactase gene promoter activity in intestinal epithelial cells. Caco-2 cells were cotransfected with lactase promoter-reporter constructs in the presence of a PDX-1 expression vector and assayed for luciferase activity. PDX-1 cotransfection results in repression of lactase promoter activity. Sequence analysis of the lactase promoter revealed a putative PDX-1 DNA binding site in the proximal 100-bp lactase gene promoter. EMSAs demonstrated that PDX-1 can interact with the lactase promoter binding site but not with a site in which the core PDX-1 binding sequence TAAT is mutated. Site-directed mutagenesis of the PDX-1 core binding site in the lactase promoter-reporter construct suggests that PDX-1 can function independently of DNA binding to its consensus binding site. Stable overexpression of PDX-1 results in repression of the endogenous human lactase gene in differentiated Caco-2 cells. Given the contrasting spatial expression pattern, PDX-1 may function to specify the anterior boundary of lactase expression in the small intestine and is thus a candidate regulator of anterior spatial restriction in the gut.

INTESTINAL lactase-phlorizin hydrolase (LPH), the absorptive enterocyte microvillus membrane glycoprotein essential for digestive hydrolysis of lactose in milk, is expressed in a spatially restricted pattern along the longitudinal axis of the gut (23). The lactase gene is expressed maximally in the proximal and middle small intestine and declines significantly in the distal segments of the intestine. Lactase gene expression is also temporally restricted in the gut during intestinal maturation. Enzyme activity is maximal in the small intestine of preweaned mammals and declines markedly during maturation. Lactase spatial restriction is regulated at the level of gene transcription, as suggested by colocalization of lactase protein and mRNA transcripts along the anterior-posterior gut axis (23). Several transcription factors [caudal-related homeobox-2 (Cdx2) protein (7, 26); GATA-4, -5, and -6 (6, 8, 16, 27); and hepatocyte nuclear factor (HNF-1) (16, 25, 27)], which are capable of activating the lactase promoter in intestinal cell culture, have been identified. However, transcription factors capable of repressing lactase promoter have not been identified.

Mechanisms regulating spatial restriction of intestine-specific gene expression, including LPH expression, along the anterior-posterior gut axis during gastrointestinal development are largely unknown. Transcription factors expressed in specific regions along the anterior-posterior gut axis during gastrointestinal organogenesis would be candidate spatial regulators. Spatial expression domains for several transcription factors expressed in endoderm-derived organs have been mapped along the anterior-posterior gut axis (10). The pancreatic duodenal homeobox-1 (PDX-1) protein (also known as insulin promoter factor 1, somatostatin transcription factor 1, and islet duodenum homebox 1) is required for pancreas development and the maintenance of functional islet β-cells (1, 15, 19–21). PDX-1 is a known activator of a number of genes essential for maintaining pancreatic cell identity and function, including insulin (22), glucose transporter 2 (29), glucokinase (30), islet amyloid polypeptide (3, 4, 24), and somatostatin (19). In addition to the pancreas, PDX-1 is expressed in the most anterior duodenal region of the intestinal tract (11) in a region in which lactase gene expression is repressed in adult mammals. Adenosine deaminase (5) and sucrase-isomaltase (13) have been identified thus far as the only intestinal target genes capable of being regulated by PDX-1. Heller et al. (13) have reported that PDX-1 is capable of repressing Cdx-2-mediated promoter activation of the sucrase-isomaltase gene. Here we investigate the ability of PDX-1 to function as a regulator of lactase promoter activity in intestinal epithelial cells in culture.

MATERIALS AND METHODS

Materials and reagents. Restriction endonucleases and modifying enzymes were purchased from New England Biolabs (Beverly, MA). Radioisotopes were purchased from PerkinElmer (Boston, MA). Oligonucleotides were synthesized in the Protein and Nucleic Acid Facility of the Stanford University Beckman Center. In-silico transcription factor binding site analysis was performed with the MatInspector software package (Genomatix, Munich, Germany).

Plasmid constructs. The rat lactase promoter-luciferase reporter plasmids pgLac100 (−100/0+13) and pgLac3k (−2,869/+13) have been described previously (6, 7) and are shown schematically in Fig. 3A. To clone the mutant lactase promoter-reporter construct pMut-2, a fragment of the pgLac100 construct was amplified by PCR using a forward mutant PDX-1 binding site oligonucleotide (Mut2 sequence shown in Fig. 1) with a 5′ BglII site added and the reverse GL primer 2. The internal RglII-Xhol fragment of the PCR product was cloned into pGL3-Basic (Promega) to generate pMut-2. Incorporation of the correct deletion and mutated base pairs was confirmed by sequencing. The PDX-1 expression plasmid, pCMV-PDX1 (also called pCMX/900), and the control empty vector pCMX were generously provided by M. Montminy (Salk Institute, San Diego, CA). The pCMV-PDX1
construct contains the rat PDX-1/STF-1 cDNA under the control of a CMV promoter. For use in the stable transfection experiments and for in vitro transcription, mouse PDX-1 cDNA, a gift of C. V. Wright (Vanderbilt University, Nashville, TN), was subcloned into pAlpha in vitro transcription, mouse PDX-1 cDNA, a gift of C. V. Wright (Vanderbilt University, Nashville, TN), was subcloned into pAlpha

**A**

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<th>PDX-1 site (Wild-Type)</th>
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**B**

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Fig. 1. Sequence of wild-type (A) and mutated pancreatic duodenal homeobox-1 (PDX-1) protein binding site oligonucleotides (Oligo) (B). A: sequence of the wild-type PDX-1 binding site probe (76 to 49) PDX-1 site is shown at top. Double-stranded mutations corresponding to bold bases were incorporated into synthetic Mut1, Mut2, and Mut3 oligonucleotides for gel shift analysis. The consensus core PDX-1 binding site is underlined. B: sequence of oligonucleotide probes spanning the 100-bp lactase promoter region with 5’ and 3’ nucleotide positions relative to start site of transcription is indicated in parentheses.

**RNA analysis by RT-PCR.** Total RNA was isolated from mouse small intestine divided into equal one-eighth segments beginning with the proximal duodenum and extending to the distal ileum using the RNaseasy mini kit (Qiagen, Valencia, CA), according to the protocol of the manufacturer. Total RNA was similarly isolated from Caco-2 cells in culture. For RT-PCR, cDNA was initially synthesized from 1.0 g of pmPDX-1 or empty vector pAlpha+ control and selected for G418 resistant colonies as previously described (7). The mouse HNF-1α expression construct pHNF-1α was generously provided by G. Crabtree (Stanford University, Stanford, CA) (17). pHNF-1α was constructed from pBJ5, which contains the SR (human T cell lymphotrophic virus type 1) promoter.

**EMSA.** The putative PDX-1 binding site probe (Fig. 1A) consisted of a double-stranded 28-nt oligonucleotide from -76 to -49 relative to the start site of transcription of the rat lactase gene (28). The radiolabeled probe was generated by annealing single-stranded oligonucleotides to yield a four-base 5’ overhang on each end followed by Klenow fragment fill-in incorporation with nucleotide triphosphates, including [32P]deoxythymidine triphosphate (3,000 Ci/mM). In vitro-translated PDX-1 was generated by using the pCMV-PDX1 linearized plasmid and the TNT Quick Coupled Transcription/Translation System (Promega). Proximal duodenal and midjejunal segments were harvested from adult FVB mice, rinsed (in 1.5 M NaCl, 1 mM diithiothreitol, 2 μg/ml aprotinin, 2 μg/ml leupeptin, 0.75 mM PMSF), everted, and scraped to isolate mucosa. Nuclear proteins were extracted from intestinal mucosa using the NE-PER kit (Pierce Biotechnology, Rockford, IL). Protein concentration was determined by the Bradford method with the Protein Assay Reagent (Bio-Rad). The PDX-1 in vitro translation reaction mixture (1.5 μl) or intestinal nuclear protein (2 μg) was incubated in the presence of 40,000 counts/min-labeled probe (106 counts/min·μg−1) with 0.5 μg poly(dI-dC) with or without 100-fold excess unlabeled competitor oligonucleotides in (in mM) 5 HEPES (pH 7.9), 25 KCl, 0.05 EDTA, 0.125 PMSF, and 10% glycerol for 20 min at room temperature. DNA-protein complexes were resolved on a 4% Tris-glycine polyacrylamide gel that was then dried and exposed to X-ray film for autoradiographic detection. For nonradioactive gel shift analysis, DNA–protein interactions were detected by using synthetic 3’ end-biotinylated probes and the LightShift chemiluminescent EMSA kit (Pierce Biotechnology, Rockford, IL). The Sp1 binding site competitor con-
The PDX-1 transcript level is maximal in the proximal duodenum and extending to the distal ileum. The pattern of PDX-1 RNA abundance contrasts with that of lactase RNA transcript was detected by RT-PCR in the distal duodenum and extending to the distal ileum. Total RNA (1.0 μg) was isolated from mouse small intestine divided into equal 1/8 segments beginning with the proximal duodenum and extending to the distal ileum. The maximal level of lactase mRNA levels were analyzed in tissue segments along the anterior-posterior gut axis.

RESULTS

Spatial patterns of lactase and PDX-1 RNA abundance along the anterior-posterior gut axis. To define the regional localization of PDX-1 and lactase along the length of the small intestine, mRNA levels were analyzed in tissue segments harvested along the length of the gastrointestinal tract. RNA was isolated from mouse small intestine divided into equal one-eighth segments beginning with the proximal duodenum and extending to the distal ileum. The maximal level of lactase RNA transcript was detected by RT-PCR in the distal duodenum and jejunum (the 3/8 to 6/8 segments) as shown in Fig. 2.

The pattern of PDX-1 RNA abundance contrasts with that of lactase RNA abundance in the anterior segments of the gut. The PDX-1 transcript level is maximal in the proximal duodenum and declines significantly in the distal duodenum and jejunum. Contrasting spatial expression patterns for the PDX-1 transcription factor with the lactase gene and the knowledge that PDX-1 is capable of repressing Cdx-2-mediated promoter activation of the sucrase-isomaltase gene (13) suggests the possibility that PDX-1 acts to repress lactase expression in the intestinal epithelial cells of the proximal duodenum.

PDX-1 is capable of repressing lactase promoter activity in intestinal cell culture. We have previously demonstrated that 3.0-kb (7) and 100-bp (6) lactase promoter fragments are capable of directing promoter activity in Caco-2 cells (12), a human adenocarcinoma-derived cell line that mimics a small intestinal enterocyte phenotype with respect to expression of several digestive hydrolases including lactase. To determine whether the transcription factor PDX-1 is capable of regulating lactase promoter activity, Caco-2 cells were cotransfected with lactase-promoter fragments and the PDX-1 expression construct, pCMV-PDX1. Caco-2 cell extracts were assayed for relative luciferase activity 48 h after transfection as shown in Fig. 3B. PDX-1 represses lactase promoter activity driven by the 3.0-kb and 100-bp promoter-reporter constructs by ~40 and 70%, respectively, relative to the empty vector control. As a control, the PDX-1 expression construct activated its known insulin promoter target in pIRES-Luc. The repression of lactase promoter activity by PDX-1 was shown to be dose dependent (Fig. 3C).

Previous studies have demonstrated that Cdx-2 (7, 26) and HNF-1 (16, 25, 27) are capable of activating the lactase promoter in cell culture by binding to distinct binding sites in the proximal promoter. To determine whether PDX-1 overexpression was capable of repressing promoter activity mediated by these factors, Caco-2 cells were cotransfected with Cdx-2 and HNF-1α expression constructs in the presence or absence of the PDX-1 expression construct (Fig. 4). Overexpression of PDX-1 resulted in complete repression of Cdx-2 and HNF-1α activation.

PDX-1 can interact with a lactase promoter DNA binding site. Inspection of the 100-bp 5′ flanking region of the rat lactase promoter reveals a consensus binding site for PDX-1 centered at nucleotide position 62 relative to the start site of transcription (Fig. 1). To identify interactions between this putative DNA binding site and PDX-1, we employed the EMSA or gel shift. The wild-type putative PDX-1 binding site oligonucleotide (PDX-1 site) was radiolabeled, incubated in the presence of in vitro transcription/translation PDX-1 extract, and then migrated through a 4% nondenaturing acrylamide gel. The autoradiograph in Fig. 5A reveals the position of the rapidly migrating unbound probe at the base of the gel and a DNA/protein complex of slower mobility, which is formed after incubation with in vitro translated PDX-1 (lane). The DNA/protein complex is not competed away by 100-fold excess, unrelated, unlabeled SP1 oligonucleotide (SP1 lane) but is competed for by 100-fold excess, unlabeled, wild-type PDX-1 site oligonucleotide (WT lane). The control antisense translated PDX-1 reaction product did not yield a specific gel shift complex (not shown).

To further define specificity of binding, we assayed the abilities of three mutant oligonucleotides to compete for PDX-1 binding to the probe. The mutant oligonucleotides differ from the wild-type PDX-1 site at clusters of three base pairs that span the length of the cis element and are shown in Fig. 1. Mutant oligonucleotides Mut1 and Mut3 provided in 100-fold excess were able to compete for protein binding, whereas Mut2 was not (Fig. 5A, compare competitor lanes). The inability of the Mut2 oligonucleotide to compete for binding suggests that the TAAT sequence is essential for PDX-1 binding. The TAAT sequence comprises the core consensus binding site for PDX-1. We confirmed PDX-1 binding to the putative binding site by incubating the DNA/protein complex in the presence or absence of a polyclonal PDX-1 antibody (Fig. 5B). The PDX-1 antibody recognized PDX-1 protein bound to the PDX-1 site probe, resulting in a complex of reduced gel mobility or a supershift. As expected, no supershift was observed for the binding reaction carried out in the presence of nonimmune serum. Gel shifts performed with
nuclear extract isolated from mouse proximal duodenum revealed a specific DNA/protein complex that was similarly competed for by mutant oligonucleotides Mut1 and Mut3 but not by Mut2 (Fig. 5C). The complex migrates with increased mobility compared with the complex generated with in vitro translated PDX-1 and does result in a supershift with PDX-1 antibody (data not shown). The intestinal nuclear protein may thus be partially degraded, despite protease inhibition, resulting in loss of antibody recognition. The DNA/protein complex was barely detectable with nuclear extract from midjejunum, consistent with reduced PDX-1 expression in the distal intestine (Fig. 5C, nuclear extract J).

To determine the regulatory effect of mutating the PDX-1 site, a mutant reporter construct (pMut-2) was generated by site-directed mutagenesis. The mutant reporter construct differs from the wild-type 100-bp lactase promoter at the base pairs corresponding to the Mut2 oligonucleotide (see Fig. 1). Cotransfection of the pMut-2 reporter construct with the PDX-1 expression construct results in a ~50% transcriptional repression, comparable to that of the wild-type promoter construct (Fig. 6). Mutation of the PDX-1 binding site therefore did not prevent repression of the lactase promoter as might have been expected. This discrepant result suggests that PDX-1 can function to repress the 100-bp lactase promoter independent of binding to the promoter candidate binding site, possibly by binding to another DNA binding site or by interacting with and inhibiting other transcriptional activator proteins. Such a mechanism for repression is consistent with a report that PDX-1 can interact with the Cdx-2 transcription factor and suppress activation of the sucrase-isomaltase gene (13). Sequence analysis of the 100-bp lactase promoter revealed only the single consensus PDX-1 binding site that we have characterized at \( \text{76 to 49.} \) To test for binding to a nonconsensus PDX-1 DNA binding site, gel shifts were performed with overlapping oligonucleotide probes spanning the region from \( \text{98 to 5 (Fig. 7).} \) The sequences from \( \text{55 to 5 (probes oligo-C and -D) did not interact with recombinant PDX-1 on gel shifts. However, in addition to oligo-B (75 to 38), which includes the consensus PDX-1 binding site, a sequence localized in the region from 98 to 64 (oligo-A) interacts with recombinant PDX-1. A faster migrating complex was also

![Fig. 3. Relative luciferase activity of intestinal cells cotransfected with lactase promoter-reporter constructs and PDX-1. A: schematic of promoter-luciferase (LUC) reporter constructs. B: proliferating Caco-2 cells were cotransfected with promoter-reporter constructs containing 3-kb (pgLac3k) or 100-bp (pgLac100) fragments of the lactase 5' flanking region cloned upstream of a firefly luciferase reporter gene in pGL3-Basic and with the PDX-1 expression construct pCMV-PDX1 or the empty vector pCMV-PDX1. Control transfection was performed with pIns-Luc. Transfection efficiencies were normalized to renilla luciferase expression of a cotransfected pRL-TK vector and expressed as fold change of luciferase activity compared with empty vector. The experiment was performed at least 3 times in triplicate (means ± SD, n = 3). C: dose-dependent PDX-1 repression of the lactase promoter. Fold change of luciferase activity of Caco-2 cells cotransfected with pgLac100 and increasing amounts of PDX-1 expression construct, pCMV-PDX1, compared with co-transfection with empty vector (means ± SD, n = 3). *Significant decrease compared with empty vector (P < 0.05).]

![Fig. 4. PDX-1 inhibits Cdx-2 and hepatocyte nuclear factor-1α (HNF) activation of the lactase promoter. Proliferating Caco-2 cells were cotransfected with the pgLac3k reporter along with pCdx-2, pHNF-1α, or pAlpha + (control) expression constructs in the presence (pCMV-PDX1) or absence (pCMX) of PDX-1. Transfection efficiencies were normalized to renilla luciferase expression of a cotransfected pRL-TK vector and expressed as relative luciferase activity (means ± SD, n = 3).]
noted and may represent increased sensitivity for a truncated PDX-1 protein using chemiluminescent detection. These results suggest that PDX-1 is capable of interacting with a nonconsensus binding site located upstream of the consensus site.

**PDX-1 overexpression represses transcription of the endogenous human lactase gene in Caco-2 cells.** To determine whether overexpression of the PDX-1 protein is capable of repressing transcription of the endogenous human lactase gene, preconfluent Caco-2 cells were stably transfected with the PDX-1 expression construct pmPDX-1 or with empty vector as a negative control. On reaching confluency in culture, Caco-2 cells differentiate and express the lactase gene. Stably transfected Caco-2 cells were therefore generated to allow for subsequent differentiation and assay of endogenous lactase expression. PDX-1 mRNA was detected by RT-PCR in the stable cell lines transfected with the PDX-1 expression construct and was undetected in cells transfected with the empty vector (data not shown). Stably transfected cells were grown to 7 days postconfluency to allow for full differentiation and assayed for lactase mRNA abundance. Caco-2 cells transfected with the PDX-1 expression construct expressed lower levels of lactase transcript compared with cells transfected with the empty vector $P < 0.0003$ (Fig. 8).

**DISCUSSION**

Spatial patterning of the GI tract during embryogenesis results in distinct expression patterns for intestine-specific genes along the longitudinal axis of the small intestine extend-
ing from duodenum to jejunum to ileum. The LPH gene is expressed maximally in the distal duodenum and jejunum with barely detectable expression in the proximal duodenum and distal ileum (23). Mechanisms regulating spatial patterning of intestinal genes, including lactase, along the anterior-posterior gut axis are largely undefined. Regulation of lactase gene transcription is likely to be mediated by complex interactions between modules of DNA cis elements (region-specific enhancers, repressors, and basal promoter elements) and intestine-specific transcription factors at discrete positions along the gut longitudinal axis and at distinct times during gut development.

We have previously demonstrated that a 2.0-kb 5′ flanking region of the lactase gene directs appropriate spatiotemporal expression of a luciferase reporter gene in transgenic mice (18). We and others (6–8, 16, 26, 27) have also characterized several transcription factors capable of activating the lactase gene promoter in intestinal cell culture. Included among these are factors that themselves are expressed in spatially restricted regions of the gut. For instance, Cdx-2 is expressed in a gradient of increasing expression extending from the jejunum to the colon (14). We hypothesize that gradients of expression of these and other key transcription factors along the anterior-posterior gut axis define the spatiotemporal restriction of lactase expression.

Contrasting mRNA abundance patterns for lactase and PDX-1 along the anterior-posterior gut axis (Fig. 2) led us to investigate whether the homedomain transcription factor PDX-1 is capable of regulating lactase gene transcription as a repressor. We speculate that maximal PDX-1 expression in the proximal duodenum (8/segment) and minimal expression of positive activators, e.g., Cdx-2 (14), result in maximal repression of lactase in that segment. Proceeding distally along the anterior-posterior gut axis as the gradient of PDX-1 expression declines and the gradient of synergistic positive activators increases, the net effect is the observed gradual increase in lactase expression. Lactase may thus be expressed at a moderate level in the two-eighths segment despite moderate PDX-1 expression because of the net influence of positive activators in the segment. In the distal half of the small intestine, similar gradients with combinations of positive and negative regulators may specify the gradual decline of lactase expression in the ileum. PDX-1 is a known activator of a number of genes essential for maintaining pancreatic cell identity and function including insulin (22), glucose transporter 2 (29), glucokinase (30), islet amyloid polypeptide (3, 4, 24), and somatostatin (19). However, only adenosine deaminase (5) and sucrase-isomaltase (13) have been identified as intestinal target genes capable of being regulated by PDX-1. In the present study, we demonstrated that PDX-1 is capable of repressing lactase gene promoter activity in intestinal cell culture (Figs. 3 and 4).

Heller et al. (13) previously reported that PDX-1 is capable of repressing Cdx-2-mediated promoter activation of the sucrase-isomaltase gene. The present study is the second report of a repressive function for PDX-1 in regulating promoter activity of an intestine-specific gene. Inspection of the lactase promoter sequence revealed a potential PDX-1 binding site. EMSAs demonstrated that PDX-1 was capable of interacting with the binding site and the core TAAT DNA sequence (Fig. 5). Interestingly, PDX-1 was capable of repressing the lactase promoter in a reporter construct in which the PDX-1 binding site was mutated (see Fig. 6). PDX-1 may thus function to repress the 100-bp lactase promoter independent of binding to the promoter candidate binding site, possibly by binding to another DNA binding site or by interacting with and inhibiting other transcriptional activator proteins. With respect to binding to another DNA site, we have shown that recombinant PDX-1 is capable of interacting with a nonconsensus sequence located in a region (−98 to −64) upstream of the consensus binding site. With respect to interacting with other transcriptional activator proteins, Heller et al. (13) demonstrated that PDX-1 binds to the transcription factor Cdx-2 and inhibits transactivation of the sucrase-isomaltase promoter by Cdx-2. PDX-1-mediated inhibition of Cdx-2 transactivation would similarly result in repression of the lactase promoter. We have shown that PDX-1 overexpression results in strong repression of the CDX-2 and HNF-1 activation of the lactase promoter (Fig. 4). Of note, the binding sites for Cdx-2; HNF-1; GATA-4, -5, and -6; and PDX-1 are all located in close proximity in the region spanning −80 to −46 bp upstream of the lactase transcription start site (16). The proximity of these nuclear proteins bound to the lactase promoter may allow for increased potential for interaction among the factors. In this regard, physical interaction between GATA-5 and HNF-1 has been shown to result in synergistic activation of the lactase promoter (27). We propose that PDX-1 may interact with transcriptional activators to result in repression of the lactase promoter. Future studies are planned to functionally characterize PDX-1 interaction with the nonconsensus binding site region (−98 to −64) and to assay for physical interaction among PDX-1; HNF-1; GATA-4, -5, and -6; and Cdx-2 at the lactase promoter.

To determine whether PDX-1 can function to repress transcription of the endogenous human lactase gene, Caco-2 cells stably expressing PDX-1 were generated. Stable transfection experiments demonstrated that overexpression of PDX-1 is also capable of repressing transcription of the endogenous human lactase gene in differentiated Caco-2 cells (Fig. 8). In support of a repressive role for PDX-1 in specifying developmental spatial restriction, Grapin-Botton et al. (9) have reported that ectopic expression of PDX-1 in chick embryo intestinal epithelial cells extinguishes markers for other nonpancreatoduodenal regions of gut endoderm. Specifically, PDX-1 expression in the small intestine between the duodenum
and yolk stalk turns CdxA off, downregulates CdxC, and turns off Hex in the bile duct. In our present study, the demonstration that PDX-1 can function to repress lactase promoter activity in vitro suggests a possible role for PDX-1 in specifying the anterior boundary of lactase gene expression in the gut. PDX-1, present at high levels in intestinal epithelial cells of the proximal duodenum, may function to repress lactase gene expression in the anterior region of the small intestine. Several intestinal digestive hydrolases, including sucrase-isomaltase, are similarly restricted along the anterior-posterior gut axis. We hypothesize that PDX-1 may function to specify the anterior boundary of expression for lactase as well as other digestive hydrolases. Future in vivo studies are planned to confirm a functional role for PDX-1 as a regulator of gut spatial restriction.

GRANTS

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REFERENCES


