Regulation of Intestine-specific Spatiotemporal Expression by the Rat Lactase Promoter

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Lactase gene transcription is spatially restricted to the proximal and middle small intestine of the developing mouse. To identify regions of the lactase gene involved in mediating the spatiotemporal expression pattern, transgenic mice harboring 0.8-, 1.3-, and 2.0-kb fragments of the 5'-flanking region cloned upstream of a firefly-luciferase reporter were generated. Transgene expression was assessed noninvasively in living mice using a sensitive low light imaging system. Two independent, 1.3- and 2.0-kb, lactase promoter-reporter transgenic lines expressed appropriate high levels of luciferase activity in the small intestine (300–5,000 relative light units/μg) with maximal expression in the middle segments. Post-weaned 30-day transgenic offspring also demonstrated an appropriate 4-fold maturational decline in luciferase expression in the small intestine. The pattern of the 2.0-kb promoter transgene mRNA abundance most closely mimicked that of the endogenous lactase gene with respect to spatiotemporal restriction. In contrast, a 0.8-kb promoter-reporter construct expressed low level luciferase activity (<25 relative light units/μg) in multiple organs and throughout the gastrointestinal tract in transgenic mice. Thus, a distinct 5'-region of the lactase promoter directs intestine-specific expression in the small intestine of transgenic mice, and regulatory sequences have been localized to a 1.2-kb region upstream of the lactase transcription start site. In addition, we have demonstrated that in vivo bioluminescence imaging can be utilized for assessment of intestinal expression patterns of a luciferase reporter gene driven by lactase promoter regions in transgenic mice.

Intestinal lactase-phlorizin hydrolase (LPH, lactase) is the absorptive enterocyte membrane glycoprotein essential for digestive hydrolysis of lactose in milk. Lactase is present predominantly along the brush-border membrane of differentiated enterocytes lining the villi of the small intestine. Expression of the lactase gene is spatially restricted along the longitudinal axis of the gut (1). 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 pre-weaned mammals and declines markedly during maturation. The maturational decline in lactase activity contrasts with a maturational increase in enzymatic activity of other intestinal hydrolases essential for digestion of solid foods (for comprehensive review see Ref. 2).

Although the mechanisms regulating the spatial and temporal restriction of lactase gene expression have not been fully defined, lactase spatiotemporal restriction is largely regulated at the level of gene transcription. This is suggested by colocalization of lactase protein along the longitudinal axis of the gut and lactase mRNA transcripts detected by Northern blot and in situ hybridization (1). With respect to regulation of the maturational decline, lactase mRNA abundance peaks prior to weaning and then declines severalfold in maturing rats (1, 3–5) and sheep (6). Krasinski et al. (4) attribute the decline in lactase mRNA abundance in rats to a decrease in transcription rate based on nuclear run-off assays. These reports support a transcriptional mechanism for regulating the lactase maturational decline. However, a lack of correlation between the decline in lactase mRNA and enzyme expression also has been described in rats (5, 7, 8), rabbits (9), pigs (10), and humans (8), suggesting that post-transcriptional mechanisms may also play a role in the maturational activity decline.

In vitro binding studies have shown that the lactase gene promoter interacts with specific nuclear proteins from intestinal cells. The enterocyte nuclear factor, NF-LPH1, binds to and protects a distinct cis element region of the pig lactase 5'-flanking region, CE-LPH1, from DNase I digestion (11). Furthermore, an electrophoretic mobility shift of CE-LPH1 is more prominent with nuclear extracts obtained from pre-weaned piglets than from adult animals. We and others (12, 13) have shown that NF-LPH1 is composed of the homeodomain protein Cdx-2 and that overexpression of the Cdx-2 protein can function to activate lactase transcription. Similarly, Fitzgerald et al. (14) have shown that the zinc finger transcription factor GATA-6 can bind to a cis element in the lactase promoter and can activate reporter gene transcription. We have recently demonstrated (15) that each of the GATA family members expressed in intestine, GATA-4 and GATA-5 in addition to GATA-6, can stimulate transcription of a reporter gene driven by the lactase promoter in intestinal cell culture.

The spatial restriction and maturational decline in lactase gene transcription is likely mediated by differential interaction between the lactase gene sequences and specific nuclear transcription factors. Regulatory DNA regions have been mapped by deletional analysis in transgenic mice for several intestine-
specific genes including calbinin-D9k (16, 17), villin (18), sucrase-isomaltase (19), intestinal fatty acid-binding protein (20–22), liver fatty acid-binding protein (23, 24), ileal lipid-binding protein (25), and adenosine deaminase (26). Troelsen et al. (27) have shown that 1 kb of the pig lactase promoter directs an intestine-specific maturation decline of the β-globin reporter gene in transgenic mice. In addition, Krasinski et al. (28) have reported intestine-specific expression in transgenic mice harboring a 2.0-kb flanking region of the rat lactase gene linked to a growth hormone reporter. In this report, we describe localization of an essential 1.2-kb region of the rat lactase gene promoter involved in directing appropriate spatial and temporal transgene expression during gut maturation.

Regulation of gene expression has been widely studied using bioluminescent markers (e.g., luciferase) as biological reporters in cell culture assays performed on cell lysates. An in vivo bioluminescent imaging method has been developed recently (29) that allows these studies to be performed in living cells and animals such that dynamic biological processes can be evaluated in intact organ systems. In this approach, the bioluminescent reporter luciferase serves as an intrinsic biological source of light that can be monitored externally providing an indication of gene expression patterns in living animal models. In this report, we have utilized a similar in vivo bioluminescent imaging method to characterize intestine-specific gene transcription patterns driven by lactase reporter-transgene lines in mice.

**EXPERIMENTAL PROCEDURES**

**Production and Identification of Transgenic Mice**—The plasmid constructs gLacE800, gLacL13k, and gLac2.Ok have been described previously (13). The constructs contain 8.5 (~819 to +13), 1.3 (~1307 to +1300), and 0.6 (~563 to +13), respectively, of the 5'-flanking sequences of the rat lactase gene cloned upstream of the firefly luciferase reporter gene in the pGL3-Basic vector (Promega, Madison, WI). Linearized and purified transgene constructs were injected into fertilized mouse eggs, reimplanted into pseudopregnant mice, and allowed to develop to term. Animals were generated through the Transgenic Core facility of the Stanford University Digestive Disease Center at the Palo Alto Medical Foundation Research Institute (Palo Alto, CA). Mice carrying transgenes were identified by PCR of genomic DNA using oligonucleotide primers specific for the rat lactase promoter (30) (GenBank™ accession number ST78539) and the firefly luciferase reporter gene (GenBank™ accession number U47295); gluc (sense), 5'-TATCCTGAGATAACCCAGTTAAA-3'; 3'-AACACACCGTGAGTGC-3', 5'-TGACACACCCAGTGAGTGC-3' (290-bp product); and lac-R, 5'-ACGAAACACCGTGAGTGC-3' (290-bp product); and lac-R, 5'-ACGAAACACCGTGAGTGC-3' (290-bp product). PCR amplification conditions were as follows: 94°C for 1 min; 55°C for 1 min; 72°C for 1 min; 30 cycles. PCR products were analyzed after electrophoresis on 2% agarose gels using a Molecular Analyzer Densitometer (Bio-Rad).

**In Situ Hybridization Analysis of Transgene Expression**—For immunohistochemical detection, mid-jejunal segments of wild-type or transgenic mice were fixed in 10% buffered formalin, embedded in paraffin, and sectioned on glass slides. Sections were then deparaffinized in xylene (2 times for 10 min) followed by sequential treatments in ethanol (100%, 80%, and 70%; 2 times for 2 min each). After washing in distilled water, slides were treated with Antigen Unmasking Solution 1:100 (Vector Laboratories, Burlingame, CA) for 2 min. Slides were then washed in PBS (3 times for 5 min) followed by a 15-min incubation in 1% H2O2 to quench endogenous peroxidase. After three PBS washes, sections were then permeabilized in 0.2% Triton X-100 in PBS for 10 min. Sections were then incubated with rabbit anti-luciferase antibody (CLONTECH, Palo Alto, CA). Sections were blocked by incubation with 10% normal goat serum, 0.3% Tween 20 in PBS for 30 min at 37°C, followed by a Biotin Block solution (Vector Laboratories) treatment according to the manufacturer. Sections were then incubated overnight at 5°C in a humid chamber with rabbit anti-luciferase antibody or normal rabbit serum diluted 1:6000 in blocking buffer. The anti-luciferase antibody was kindly provided by Dr. Hans Just, Institute for Biochemical and Biophysical Research, University of Hamburg, Germany (34, 35). Following antibody incubation, sections were washed in 0.3% Tween 20, PBS (5 times for 10 min each) and then incubated at room temperature for 60 min with biotinylated goat anti-rabbit IgG diluted 1:2000 in 1% normal goat serum, 0.3% Tween 20. Detection of the antigen-antibody complex was performed with the Vectastain ABC kit (Vector Laboratories) avidin/biotin method according to the manufacturer’s instructions. Briefly, sections were incubated with the avidin/biotin/horseradish peroxidase reagent, washed in PBS, reacted with fresh 3,3-diaminobenzidine for 2 min, washed in distilled water, and counterstained with hematoxylin.

**In Situ Hybridization Analysis of Transgene Expression**—For in situ hybridization, gene-specific RNA was detected in mid-jejunal sections from wild-type or transgenic mice using biotin-labeled RNA probes. For luciferase detection, a 0.7-kb fragment (NcoI-SphI) of the full-length luciferase gene was cloned from pGL3-Basic into pbLueScript KSII (Stratagene, La Jolla, CA). For lactase detection, a 0.5-kb fragment (SacI-HindIII from exon 1 of the rat lactase gene) was cloned into pbLueScript KSII. Biotin-labeled sense and antisense luciferase and lactase RNA probes were prepared, and in situ hybridization was performed using the protocols in situ Hybridization Labeled Probes (Sigma). In brief, sections were prepared as above with the exception that the jejunal segment was flushed with RNAlater (Ambion, Austin, TX) prior to formalin fixation. After deparaffinization, sections were incubated with 3% hydrogen peroxide, treated with proteinase K (30 µg/ml) for 10 min at 37°C, washed in PBS, and then dehydrated in graded ethanol treatments. The sections were hybridized
RESULTS

Generation of Lactase-Luciferase Fusion Transgenic Mice—5'-Flanking regions of the rat lactase gene cloned upstream of a firefly luciferase reporter gene were microinjected to generate transgenic founder mice (Fig. 1). Transgenic mice were identified by genomic PCR detection and confirmed by Southern hybridization. The gLac800 transgene, containing the proximal 800 bp of the lactase 5'-flanking region in the pGL3-Basic reporter vector, was incorporated into seven founder LacLuc-0.8k mice. The gLac1.3k transgene, containing 1.3-kb of the lactase 5'-flanking region was incorporated into two founder LacLuc-1.3k mice. The gLac2.0k transgene, containing 2.0-kb of the lactase 5'-flanking region, was incorporated into four founder LacLuc-2.0k mice. The promoterless vector, pGL3-Basic, and the SV40 promoter/enhancer construct, pGL3-Control, were each incorporated into a founder mouse as controls. The number of transgenes integrated into the DNA of each founder mouse varied from 1 to 33 copies/cells (Fig. 1).

Transgene expression was detected by assaying for luciferase activity in organ homogenates. Four of the seven LacLuc-1.3k lines and two of the four LacLuc-2.0k lines expressed detectable levels of luciferase. Each of the pGL3-Basic and pGL3-Control transgenic mice expressed the luciferase reporter gene.

An in vivo bioluminescence imaging method was used to rapidly screen for transgenic reporter expression in founders and subsequent F1 offspring. Specifically, luciferase substrate was injected intraperitoneally into sedated mice, and light emission was detected with a low light imaging system using an ICCD camera. A representative pseudocolor image depicting photon emission intensity used to identify rapidly the transgenic versus non-transgenic F1 generation offspring of the LacLuc-2.0kA founder line is shown in Fig. 2. Transgenic mice expressing the luciferase reporter were readily detected at 7 days of life (Fig. 2A) and in the same animals at 28 days (Fig. 2B). The results of in vivo screening were confirmed by PCR analysis of genomic DNA and by quantitative luciferase assay of internal organ homogenates by luminometer. The non-transgenic (PCR-) F1 littermates do not express luciferase and do not emit light. Blue illustrates the least intense and red the most intense light emission signals.

FIG. 1. Transgenic constructs and founder mice. Transgenic mice were established with the luciferase reporter constructs shown in the schematic. 0.8-, 1.3-, and 2.0-kb of the 5'-flanking region of the rat lactase gene were cloned upstream of the firefly luciferase reporter gene in pGL3-Basic to generate gLac800, gLac1.3k, and gLac2.0k, respectively. Transgenic mice were also generated with the promoterless vector, pGL3-Basic, and the SV40 promoter/enhancer construct, pGL3-Control. In the table, the number of transgenic mice founders are indicated in parentheses for each transgenic construct. Founders expressing detectable levels of luciferase activity are identified in the column labeled Luciferase Expressing Lines with transgene copy number in the adjacent column.

FIG. 2. In vivo photonic imaging to detect transgenic reporter expression. Luciferin was injected intraperitoneally into sedated mice, and light emission was detected with an intensified charge-coupled device camera. F1 offspring of the LacLuc-2.0kA transgenic mouse were imaged at 7 (A) and 28 days of life (B). Transgenic (PCR+) mice that emit light localized to the abdomen are shown in blue. The non-transgenic (PCR−) F1, littermates do not express luciferase and do not emit light. Blue illustrates the least intense and red the most intense light emission signals.
small intestine. The “promoterless” pGL3-Basic transgene expressed low level luciferase activity (≤5.0 RLU/μg) in multiple organs. The pGL3-Control transgene that bears an SV40 promoter-enhancer linked to luciferase reporter directed low levels of expression in multiple organs with slightly higher expression in the heart (20 RLU/μg).

To confirm that the expression pattern directed by the 1.3- and 2.0-kb 5′-flanking sequences represents tissue-specific transcription, the luciferase activity quantified above was compared with the transcript abundance for the lactase reporter gene mRNA and the endogenous lactase mRNA (Fig. 3B). Total RNA was isolated from intestinal and non-intestinal tissues of LacLuc-1.3kD and LacLuc-2.0kA transgenic mice and wild-type mice and analyzed for gene transcription by RT-PCR analysis. The tissue-specific pattern of luciferase enzyme activity correlates with luciferase mRNA abundance detected by RT-PCR. There was significantly enhanced transgene expression in the small intestine (compare Fig. 3, A and B). In addition, transcription from the 1.3- and 2.0-kb 5′-flanking segments of the lactase-luciferase transgene correlates with the pattern of transcription of the endogenous lactase gene (Fig. 3B). Specifically, RT-PCR detected high levels of lactase and luciferase mRNA in the jejunum, and transcript levels were below detectable levels in the stomach, colon, and non-intestinal organs. Luciferase activity and mRNA was detected in the liver of the LacLuc-1.3kD line and may thus indicate the absence of a liver-specific repressor present in the larger 2.0-kb construct. The minus reverse transcriptase control amplifications were negative in all organs (data not shown).

**Spatial Restriction of LacLuc Transgene Expression Along the Length of the Intestine—**To define further the regional expression of the lactase promoter-reporter transgene along the gut longitudinal axis, luciferase activity and mRNA levels were analyzed in tissue segments harvested along the length of the gastrointestinal tract. The small intestine from mice of the LacLuc-2.0kA and LacLuc-1.3kD lines was divided into equal one-eighth segments beginning with the proximal duodenum and extending to the distal ileum. The maximal level of reporter expression was detected by luciferase activity in the distal duodenum and jejunum (the 3/8th to 6/8th segments) as shown in Fig. 4A (LacLuc-2.0kA) and Fig. 4C (LacLuc-1.3kD). The pattern of luciferase activity correlated with luciferase mRNA levels as detected by RT-PCR with maximal levels of transgene transcription and protein expression in the 3/8th to 6/8th segments (Fig. 4, B and D). This region-specific transcription pattern directed by two independent 5′-flanking region transgenes corresponds closely to the regional transcription (distal duodenum and jejunum) of the endogenous lactase gene (compare lactase and luciferase panels). The LacLuc-2.0kA transgene expression zone closely approximates the endogenous lactase expression zone. The LacLuc-1.3kD transgene expression pattern overlaps with the endogenous zone and extends to slightly more distal segments of the small intestine.

Detection of bioluminescence along the gastrointestinal tract using the ICCD camera allowed direct visual confirmation of the spatial restriction of the lactase-luciferase transgene (Fig. 5). The gastrointestinal organs from an adult LacLuc-2.0kA mouse were harvested en bloc and bathed in a buffered luciferin solution and imaged with the ICCD camera. Maximal light emission was detected in the proximal-middle segment of the small intestine.

5′-Flanking Sequences of the Rat Lactase Gene Drive a Maturational Decline in Transgene Transcription—Lactase gene transcription declines dramatically at around the time of weaning, between 2 and 3 weeks in mice. To determine whether the lactase-luciferase transgenes were capable of directing a similar maturational decline, the mid-jejunum was harvested from LacLuc-2.0kA transgenic littersmates sacrificed at 1, 2, and 4 weeks of age. Transgene expression was quantified by measuring luciferase enzyme activity and transcript abundance. For the LacLuc-2.0kA mouse line, luciferase activity declined ~6-fold during this time (Fig. 6A). Luciferase reporter gene mRNA levels underwent a similar decline, which again correlated with that of the endogenous lactase gene message (Fig. 6B). These results suggest that the 2.0-kb flanking region possesses regulatory elements capable of directing the temporal decline in lactase gene transcription as well.

**Cell-specific Transcription of the LacLuc Reporter Transgene—**The small intestine is composed of a layer of mesenchymal cells surrounding an inner epithelial cell layer. The endogenous lactase gene is expressed in the enteroocyte epithelial cells lining the villi of the distal duodenum and jejunum. To localize cells expressing the luciferase reporter transgene, both immunohistochemistry and in situ RNA hybridization were performed on sections of the small intestine of the LacLuc-2.0kA mice or FVB wild-type controls. Luciferase antibody binding localized transgene reporter gene expression to epithelial cells lining the villi in jejunal sections from LacLuc-2.0kA mice (Fig. 7, A and B). Maximal staining was detected at the tips of the villi with no detectable staining in the crypts. Luciferase staining was specific to the transgenic mice, since the luciferase antibody did not bind to sections from non-transgenic wild-type mice (Fig. 7, D and E). Control reactions per-
formed on transgenic sections using normal serum as control resulted in no detectable staining (Fig. 7G).

Similar to the luciferase antibody, a biotin-labeled luciferase antisense probe hybridized predominantly in a cytoplasmic distribution to the epithelial cells lining the villi (Fig. 7C). There was no detectable hybridization of the luciferase antisense probe to jejunal sections from wild-type mice (Fig. 7F). Hybridization with the control luciferase sense probe showed no detectable background staining (Fig. 7I). As a positive control, endogenous lactase RNA was also localized to the cytoplasm of villus epithelial cells by in situ hybridization (Fig. 7H).

**DISCUSSION**

The mechanisms regulating the spatial and temporal patterns of intestinal lactase gene expression have not been fully defined. Characterization of the lactase promoter in intestinal cell culture has led to the identification of several nuclear proteins involved in regulating intestine-specific transcription including Cdx2 (12, 13), GATA-4/5/6 (14, 15), and HNF-1 (36, 37). The characterization of these regulatory elements and factors has been studied predominantly in cell culture with two previous exceptions. Troelsen et al. (27) reported that a 1-kb flanking region of the pig lactase gene directed a region-specific pattern of expression in transgenic mice. The transgene also resulted in a maturational decline that closely correlated with the endogenous lactase gene. Krasinski et al. (28) reported that a 2.0-kb promoter transgene resulted in an increase in reporter transcription during times when there was a decrease in transcription of the endogenous gene (28). These studies provided evidence that important spatial temporal control elements were likely to be located in the 5' flanking sequence of the lactase gene. We have reported previously (13) that 2.0 kb of the 5' flanking region of the rat lactase gene linked to the luciferase reporter gene was capable of directing a 4–6-fold increase in reporter expression over a “promoterless” vector in transiently transfected intestinal cell culture. The 800-bp flanking region sequences possessed 50% of the 2.0-kb promoter activity in cell culture trans-
We have now generated transgenic animals harboring the two constructs and have observed a differential pattern of organ-specific expression.

In vivo and ex vivo methods were capable of detecting luciferase signals emitted from the transgenic reporter expressed by intestinal epithelial cells of transgenic mice. The 800-bp flanking sequences direct a low level of luciferase activity in several intestinal as well as non-intestinal organs similar to a transgenic line harboring the promoterless pGL3-Basic and the SV40 promoter/enhancer pGL3-Control. The pGL3-Basic construct results in a high background reporter activity in intestinal cell culture and likely contains cryptic promoter activity also present in the transgenic line. Whereas the 800-bp fragment directed a low level luciferase expression in multiple organs, the 2.0-kb flanking sequences directed high level reporter expression restricted to the intestine, and the 1.3-kb fragment directed intestine and liver expression (Fig. 3). Along the rostrocaudal axis of the small intestine there was spatial restriction of transgene expression for the two independent 2.0- and 1.3-kb lactase promoter transgenic lines. Maximal expression was noted in the proximal and middle segments of the small intestine (Fig. 4). The pattern of expression was also represented in transgene mRNA transcript abundance in the intestinal segments. The spatial restriction of transcription corresponded most closely with the spatial restriction of the endogenous lactase gene in the LacLuc-2.0k mice. These results imply that tissue-specific enhancer elements likely reside in the 2.0-kb flanking sequence. This finding is consistent with the report of tissue-specific expression with a similar 2.0-kb flanking region clone linked to a growth hormone reporter described by Krasinski et al. (28). However, the pattern of transgene expression in the LacLuc-1.3k and −2.0k mice described in our report appears to more closely mimic the intestinal expression pattern of the endogenous gene. Differences in the transgene expression patterns in the two reports may be due to positional integration and copy number effects. The absence of high level intestinal expression in the 800-bp transgenic lines suggests that the tissue-specific enhancer for the rat lactase gene may be in the region between −800 and −2000 of the flanking region. As described above, Troelsen et al. (27) were able to detect intestine-specific expression with a construct bearing the proximal 1.0-kb of the pig promoter. Sequence comparison, however, between the pig and rat lactase promoter reveals no significant homology outside of the proximal 200-bp sequence. It is therefore likely that tissue-specific enhancer elements for the lactase gene for different species will be located in different regions. In addition, for the LacLuc-1.3kD mice, the presence of an expanded zone of transgene expression along the rostrocaudal gut axis and ectopic transgene expression in the liver may result from absence of ileal-specific or liver-specific repressive elements that are present in the larger 2.0-kb promoter fragment.

The proximal 2.0-kb flanking sequence of the rat lactase

![Fig. 6. LacLuc-2.0k transgene expression during gut maturation.](image1)

Mid-jejunal sections from a LacLuc-2.0k transgenic mouse (A and B) and a non-transgenic FVB mouse (D and E) immunoperoxidase-stained (brown) for luciferase and counterstained with hematoxylin. G, absence of cell-specific staining in the LacLuc-2.0kA section using nonimmune serum. In situ hybridization analysis of mid-jejunal sections from a LacLuc-2.0k mouse (C) and a non-transgenic FVB mouse (F) hybridized with a biotinylated luciferase antisense RNA probe. J, absence of cell-specific staining in the LacLuc-2.0kA section hybridized with the luciferase sense RNA probe. H, positive control, villus epithelial cell staining of the LacLuc-2.0kA section hybridized with a lactase antisense RNA probe. Magnification ×40 (A, B, and C) and ×100 (all others). m, muscularis; c, crypt; v, villus.
gene also directs cell-specific expression within the small intestine. The small intestine is composed of a layer of mesenchymal cells surrounding an inner epithelial cell layer. Lactase gene transcription is localized to the absorptive epithelial cells lining the villus and is maximal in the distal villus cells. In jejunal sections from the lactase promoter-luciferase reporter transgenic mice, luciferase expression was appropriately localized to the villus epithelial cells as detected by immunohistochemical staining and in situ hybridization (Fig. 7). The 5′-flanking sequences of the 2.0-kb LacLac transgene also direct a temporal decline in luciferase expression during maturation in the transgenic animals. Luciferase reporter activity and transcript abundance for the 2.0-kb flanking sequence transgene was increased in all intestinal segments in 7-day-old mice and declined 6-fold by 28 days in post-weaned adults (Fig. 6). Again this temporal restriction of transgene expression is closely correlated with the maturational decline observed for the endogenous lactase gene. These results imply that key maturational control elements may reside within this 2.0-kb flanking region. Localization of such elements to this region is consistent with the finding of a similar maturational decline in reporter gene expression driven by a 1-kb flanking region pig lactase transgene (27). As described above, there is a significant sequence homology between the pig and rat lactase genes within the proximal 200-bp region. It is possible that the maturational decline element is located in this conserved region. Within this sequence there are several Cdx-2-binding sites. The proximal Cdx-2-binding site, CE-LPH1, is reported to bind nuclear protein more abundantly in nuclear extract from pre-weaned compared with post-weaned adult pigs (11). Such a differential interaction between this element and a maturation-specific nuclear factor may be involved in regulating the decline in lactase transcription associated with weaning.

Screening of neonatal founders and offspring expressing the lactase-luciferase transgenes was greatly facilitated by in vivo detection of luciferase activity with an ICCD camera. In vivo imaging has been employed previously to detect luciferase reporter expression by bacteria in the digestive tract (38). In our report, in vivo imaging was capable of detecting luciferase signal emitted from the transgenic reporter expressed by intestinal epithelial cells of transgenic mice (Fig. 2). Rapid noninvasive detection of light emitted from the living mice allowed for analysis of reporter gene expression in less time and using fewer animals. Of broader significance is the ability to easily assay a gut epithelial cell marker (exogenous luciferase) both in vivo and ex vivo in the LacLac mice. Extensive research in gut development, oncology, infectious disease, and gene therapy has been directed toward the study of intestinal epithelial cell response to biological or chemical stimuli. Examples include the gut recovery from enteroviral infection, hypoxic ischemia, radiation, or chemotherapeutic injury. In vivo or ex vivo measurement of luciferase expression in intestinal epithelial cells may be useful in rapidly assessing dynamic changes such as these and other physiological changes in the gut.

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