Sucrase-isomaltase (SI), an intestine-specific gene, is regulated by homeodomain proteins Cdx2 and GATA-4 during the suckling-weaning transition in mice. We have previously identified cis-acting elements within a short evolutionarily conserved SI promoter. However, the nature and profile of expression of the interacting proteins have not been fully characterized during this developmental transition. Herein, we show that hepatocyte nuclear factor-1α (HNF-1α), GATA-4, and caudal related homeodomain proteins Cdx2 and Cdx1 are the primary transcription factors from the adult mouse intestinal epithelium to interact with the SIF3, GATA, and SIF1 elements of the SI promoter. We wanted to study whether HNF-1α, GATA-4, and Cdx2 can cooperate in the regulation of SI gene expression. Immunolocalization experiments revealed that HNF-1α is detected in rare epithelial cells of suckling mice and becomes progressively more expressed in the villous epithelial cells during the suckling-weaning transition. GATA-4 protein is expressed exclusively in villous differentiated epithelial cells of the proximal small intestine, decreases in expression in the ileum, and becomes undetectable in the colon. HNF-1α, GATA-4, and Cdx2 interact in vitro and in vivo. These factors activate SI promoter activity in cotransfection experiments where GATA-4 requires the presence of both HNF-1α and Cdx2. These findings imply a combinatorial role of HNF-1α, Cdx2, and GATA-4 for the time- and position-dependent regulation of SI transcription during development.

The maturation of the intestinal epithelium involves several steps that are initiated early during embryogenesis and completed at the beginning of adulthood (1, 2). In mice, important changes in the acquisition of mature enterocyte functions are observed during the 3rd postnatal week, which corresponds to the suckling-weaning transition. Among these changes are the activation of transcription of distinct genes related to specific functions of the intestinal epithelium (3). Sucrase-isomaltase (SI), an enzyme expressed in the brush border of mature enterocytes, represents one of the most extensively studied genes activated during this developmental transition (4). SI is first detectable in mouse embryonic intestine and remains stable through the first 2 weeks of postnatal life. At the time of the suckling-weaning transition, a dramatic induction of SI mRNA and protein is observed. A proximal to distal gradient of SI expression and activity is then established with highest level in the jejunum and no detectable level in the colon (3, 5, 6). A vertical gradient of expression is also observed along the crypt-villus axis with a maximal level in the lower two-thirds of the villi and no expression in the crypts (3, 7, 8). Although the distribution of SI expression is well documented during intestinal development, the exact developmental mechanisms that govern SI and intestinal gene expression are still poorly defined.

In the past, we have identified a number of SI promoter elements important to support its expression in intestinal epithelial cells (9). Furthermore, a short evolutionarily conserved SI gene promoter was shown to recapitulate the developmental expression of SI in transgenic mice (6). This indicated that all of the elements required for the developmental regulation of the SI gene are comprised within this short region. This short promoter contains three identified regulatory sites: sucrase-isomaltase footprint 1 (SIF1), SIF3, and GATA.

The SIF1 element interacts with Cdx transcription factors (10, 11) that belong to mammalian homeobox gene family related to the Drosophila melanogaster gene caudal (12). The expression of Cdx1 and Cdx2 is restricted to the intestinal epithelium (13). Cdx1 protein is found mainly in the crypt compartment, whereas the Cdx2 protein is detected in both the...
crypt and the villus compartments (13–15). Cdx2 stimulates differentiation and expression of SI in intestinal epithelial cells (16). However, the specific role of the SIF1 element in the regulation of SI promoter activity in vivo is unclear.

The SIF3 element interacts with the transcription factors HNF-1α and HNF-1β, which bind to DNA as either homodimers or heterodimers. They are expressed in a variety of tissues, including liver, kidney, and intestine and have been implicated in the regulation of multiple genes (17). HNF-1α stimulates SI promoter activity via the SIF3 element, whereas HNF-1β down-regulates this effect (18). Although HNF-1α mRNA has been localized in the crypts and bottom of the villi in the small intestine (19), the exact pattern of HNF-1α protein distribution during intestinal development is unknown.

More recently (20, 21), we have identified a GATA element localized in the proximal region of the SI promoter. GATA-4, 5, and 6 are expressed in the intestine as well as various mesoderm- and endoderm-derived tissues such as heart, liver, lung, and gonad, where they play critical roles in regulating tissue-specific gene expression (22). GATA-4 transcripts are detected in villi of the small intestine in both mice (23) and chicken (24), whereas the GATA-6 mRNA is localized predominantly at the crypt-villus junction (24). However, the exact distribution of GATA proteins in the intestine has not been addressed. GATA-4 and GATA-6 play critical roles in the definitive formation of the endoderm early during embryonic life (25–28). Their involvement in the maintenance of the intestinal epithelium remains speculative.

In this study, we investigated whether HNF-1α, GATA-4, and Cdx2 could activate the development-regulated SI gene through combinatorial interactions. We report that HNF-1α and GATA-4 protein expression is temporally and regionally modulated in a way that correlates well with the activation of SI expression during the suckling-weaning transition in the intestine. We show that these proteins physically interact with each other and cooperate functionally in the activation of the SI gene promoter. These findings imply a combinatorial role of HNF-1α, Cdx2, and GATA-4 for the maturation of the small intestinal mucosa.

Experimental Procedures

Plasmid Construction, Mutagenesis, and Transgenic Mice—The −201 to −54 murine SI (mSI)-luciferase and −134 to +40 intestinal phospholipase A/lyso phospholipase (IPAL)-luciferase constructs have been described elsewhere (18, 29). Point mutagenesis of the SI promoter was performed using a PCR-based mutagenesis approach (30). The following oligonucleotides were designed to create point mutations (underlined) of the specific elements: SIF1m (sense) 5′-GTTGAAAGTGCAGGCAACGGGATGATGATC3′ and SIF1m (antisense) 5′-TTTCTACTACTCCGTTGACCTGACCTTAC3′; GATAm (sense) 5′-TAAACAGTGATGGCCTGGTAGGAAG-3′ and GATAm (antisense) 5′-GGTTCTCAACAGGGTGATGAA-3′. Integrity of the mutant constructs was confirmed by sequence analysis. The −8.5 to +54 SIF1mSHGH plasmid was constructed and injected to produce transgenic mice as described previously (18). Cdx2 transgenic mice were generated as described elsewhere (18, 29). Point mutagenesis of the SI promoter containing 10% fetal bovine serum. The luciferase and Renilla activities were determined 48 h after transfection using the dual luciferase assay kit (Promega Biotech, Madison, WI). Each experiment was repeated at least three times in triplicate. For stable transfections, Caco-2/15 subclone cells were trypsinized 48 h after transfection and maintained in selection medium containing 1 mg/ml G418.

RNA Analysis—RNA was extracted from multiple tissues or from cultured cells, and ribonuclease (RNase) protection analyses were performed as described previously (18). Ribonuclease promoter for the detection of m36B4, h36B4, and m36B4 mRNA were prepared as described previously (18).

Immunohistochemistry—Immunohistochemistry was performed exactly as described previously (13, 15). After blocking with protein-blocking agent (Coulter-Immunotech, Miami, FL), the slides were incubated with primary antibody as detailed in the figure legends. The following antibodies from Santa Cruz Biotechnology (Santa Cruz, CA) were used: anti-goat GATA-4 (SC-1237) at a concentration of 0.2 μg/ml; anti-rabbit GATA-4 (H-112) at a concentration of 0.2 μg/ml; anti-goat HNF-1α (SC-6547) at a concentration of 0.067 μg/ml. Cdx2 protein was detected with affinity-purified CNL antibody (1:1,000) (15). All primary antibodies were visualized with a biotinylated secondary antibody and alkaline phosphatase detection system. The slides were developed with nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate (Roche Molecular Biochemicals). The tissue was lightly counterstained with 2% neutral red and mounted with Permount (Fisher Scientific).

Immunohistochemistry was repeated at least three times for each antibody with sections obtained each time from different mice. Each antibody was separated by 10 and 15 days to prevent any modification of expression related to the circadian rhythm as reported for HNF-1 proteins (33). Specificity of the signal for each antibody was confirmed by blocking experiments as described previously (13).

Isolation of Nuclear Proteins from Adult Intestinal Epithelium—Nuclear protein was isolated from the intestinal epithelium of adult mice by an adaptation of a method described previously which used human intestine (34). Briefly, mice were sacrificed, and the intestine was separated in sections of jejunum, ileum, and proximal colon. Each section was opened longitudinally and rinsed with cold PBS. The sections were further cut in 5-mm pieces and incubated in 5 ml of cold MatriSperse (Becton-Dickinson) in 15-ml tubes at 4 °C for 18–24 h. The epithelial layer was dissociated by gentle manual shaking. The epithelial suspension was collected, centrifuged, and washed with cold PBS. Nuclear proteins were then isolated from the epithelial cell pellet as described previously (9).

Western Blot Analysis—20 μg of nuclear protein extracts was analyzed by a 4–12% BisTris NuPAGE (Invitrogen) and transferred to an Immobilon-P membrane. Western blotting was then performed exactly as described previously (18). The following antibodies from Santa Cruz Biotechnology (Santa Cruz, CA) were used: HNF-1α affinity-purified rabbit polyclonal antibody raised against a polyclonal antibody raised against a peptide mapping to the amino terminus of murine Cdx2 (CNL) was used for Cdx2 detection (15).

Electrophoretic Mobility Shift Assay (EMSA)—EMSA was performed essentially as described previously (10). The reactions were performed in 20 μl of binding buffer (10) containing 5 μg of nuclear extracts, 1 μg of poly(dIdC), and 20,000 cpm of 32P-labeled DNA probe for 30 min. Complexes were then separated on a 4% polyacrylamide gel at room temperature (SIP1 and SIP3) or 4 °C (GATA), dried, and exposed on a PhosphorImager screen. The following double-stranded oligonucleotides were used as DNA probes: GATA, 5′-AACACATTGATGACCTTTGTGA3′; MUT-GATA, 5′-AACACATTGATGACCTTTGTGA3′; HNF-1α, 5′-GATCTCGTCACAAACGGTGATGAA-3′; GATA-4, 5′-TTTCTACTACTCCGTTGACCTGACCTTAC3′; and GATA-6, 5′-GGTTCTCAACAGGGTGATGAA-3′. The SIF1 and SIP3 DNA probes have been described previously (9). When indicated, antibodies were included in the binding reaction for supershift analysis.

GST Pulldown—GST-protein interaction assays were performed essentially as described elsewhere (35). Briefly, bacterial expression of GST fusion proteins was induced in medium containing 0.1 μM isopropyl-1-thio-galactopyranoside for 3 h. The bacterial pellet was washed twice in PBS containing 1 mM phenylmethylsulfonyl fluoride to disrupt the bacteria. The bacterial residues were removed by centrifugation, and the supernatant was incubated with GST beads (Amersham Biosciences) on a rotating wheel for 30 min. The beads were washed six times with PBS and the GST fusion proteins eluted with 10 mM reduced glutathione according to the manufacturer’s instructions. Reduced glutathione was removed by dialysis in dialysis buffer (0.1 M...
Developmental Intestinal Expression of HNF-1α, GATA-4, Cdx2

RESULTS

**HNF-1α, GATA-4, Cdx2, and Cdx1 Interact with the SI Promoter in Adult Mouse Enterocytes**—The SI gene promoter contains several cis-acting elements important for transcriptional regulation (9) (Fig. 1A). The identity of proteins able to interact with these elements has been investigated previously with the use of intestinal cell lines. We wanted to confirm the nature of proteins of the mouse intestinal epithelium susceptible to interact with the SIF3, GATA, and SIF1 elements.

Because the SI gene is highly transcriptionally active in the adult small intestinal epithelium, nuclear extracts were prepared from adult mouse isolated enterocytes, incubated with a SIF3, GATA, or SIF1 32P-labeled probe, and migrated on a polyacrylamide gel. As reported previously, the SIF3 probe produced a single complex that was supershifted when an HNF-1α polyclonal antibody was included in the binding reaction (Fig. 1B) (18). The GATA probe produced an intense complex that was mostly supershifted with the addition of a GATA-4 polyclonal antibody (Fig. 1C, upper panel). The addition of GATA-5 or GATA-6 polyclonal antibodies did not supershift this complex (Fig. 1C, upper panel). Control experiments performed with nuclear extracts prepared from GATA-5- and GATA-6-transfected COS-7 cells confirmed the ability of both antibodies to supershift a GATA-specific complex (Fig. 1C, lower panel). This suggested that GATA-4 was the main protein in adult intestinal epithelium to interact with the SI-GATA site. Incubation of intestinal nuclear extracts with the SIF1 probe produced two very close migrating complexes (Fig. 1D). Supershift analysis showed that both Cdx1 and Cdx2 complexes were present and separable by size (Fig. 1D).

**Mutagenesis of the SIF1 Element Abolishes SI Promoter Activity in Vivo**—The role of SIF3 and GATA elements in the regulation of SI promoter activity in vivo has been characterized previously (18, 20). The SIF1 element is crucial for high level of SI promoter activity in vitro (9, 10). To evaluate the importance of the SIF1 element in the regulation of the SI gene in the intact mouse intestinal epithelium, we designed a transgenic construct harboring point mutations within this element and linked nucleotides −8.5 kb to +54 of the mSI gene to the hGH reporter gene (Fig 2A). The wild-type construct supports hGH expression in enterocytes of transgenic mice (18, 32), and the SIF1 mutation abolishes in vivo interaction of Cdx proteins (10). The effect of the SIF1 mutation on hGH expression in two transgenic lines was compared with the previously characterized wild-type (WT) F0 (17) F1 (32) transgenic line. Total RNA isolated from different portions of the intestine was analyzed by an RNase protection assay. Each RNA sample was incubated with probes for hGH and an internal control (36B4) in the same hybridization solution to correlate hGH mRNA levels.
among these different lines. The WT transgenic line showed high level expression of hGH in the small intestine (Fig. 2B), as reported previously (32). In contrast, no detectable level for hGH mRNA was found in the intestine of the SIF1 mutant transgenic lines (Fig. 2B). To analyze further the importance of the SIF1 element in regulating SI promoter activity in vivo, we utilized the Caco-2/15 cell line that spontaneously differentiates in postconfluence with concomitant high induction of SI expression. The WT and the SIF1 mutant constructs were cotransfected with a neomycin resistance expression vector (pRC/CMV) to stably transfect Caco-2/15 cells. As a control, Caco-2/15 cells were cotransfected with the empty hGH reporter and pRC/CMV. Expression of hSI, hGH, and h36B4 was determined in RNA samples isolated from neomycin-resistant population of clones at different times of cell confluence. Expression of the WT construct increased in a manner similar to endogenous human SI mRNA during cellular differentiation (Fig. 2C and D). In contrast, the SIF1 mutant construct...
showed a stable level of hGH expression during differentiation (Fig. 2, C and D) and was comparable with the level detected in Caco-2/15 stably cotransfected with the empty vector (Fig. 2, C and D). These results indicate that the SIF1 element is essential to activate the SI promoter in vivo.

The Combined Temporal and Spatial Distribution of HNF-1α, GATA-4, and Cdx2 Correlates Well with the Induction of SI Expression during the Suckling-Weaning Transition—Although our results demonstrate that the SIF1 element is essential for SI gene transcription in enterocytes, it is unlikely that Cdx proteins alone are responsible for the strong induction of SI expression during the suckling-weaning transition. Indeed, the pattern of Cdx2 protein expression remains primarily unchanged throughout postnatal development, and the Cdx1 protein is found mainly in the crypt compartment where SI is not expressed (13). Because HNF-1α and GATA-4 interact with the SI promoter (Fig. 1), we investigated the expression profile of these proteins by immunohistochemistry from postnatal days 12 to 21. This transition period reflects the major changes observed for SI gene expression (6). The Cdx2 protein distribution was monitored as a reference control. At postnatal day 12, a period of time when SI is undetectable in the small intestine, the HNF-1α protein was detected in the nucleus of very rare enterocytes of the jejunum (Fig. 3A, upper panel). This pattern was observed throughout the entire small intestinal tract (data not shown). In contrast, HNF-1α was detected in the nucleus of mostly all epithelial cells of the proximal colon (Fig. 3A, lower panel). This pattern was observed throughout the entire small intestinal tract (data not shown). At postnatal day 15, HNF-1α became detected in the nucleus of villus enterocytes of the small intestine as well as in the surface colonocytes of the proximal colon with the use of the CNL antibody, which recognizes the unphosphorylated form of the serine 60 residue of Cdx2 (15) (Fig. 3C).
enterocytes localized mainly at the crypt-villus junction in the jejunum, whereas the pattern in the proximal colon remained the same as postnatal day 12 (Fig. 3D). Cdx2 was restricted to the nucleus of villus enterocytes in the jejunum (Fig. 3E, upper panel) and remained undetectable in the colon (Fig. 3E, lower panel). Cdx2 remained unchanged compared with day 12 (Fig. 3F). At postnatal day 17, HNF-1α and GATA-4 were detected primarily in all enterocytes of the villus compartment in the jejunum (Fig. 3, G and H) and the ileum (not shown), whereas the Cdx2 pattern remained unchanged compared with previous time points (Fig. 3I). GATA-4 remained undetectable in the colon (Fig. 3H, lower panel). From postnatal day 21 to adulthood, HNF-1α was detected along the entire axis of the small intestine in abundance in the nucleus of villous enterocytes with only rare positively stained nuclei in crypt epithelial cells (Fig. 3J). GATA-4 protein was detected in the nucleus of villous epithelial cells of the entire small intestine (Fig. 3K, upper panel) with no detectable signal in the colon (Fig. 3K, lower panel). Cdx2 localization was comparable with the earlier time points during development (Fig. 3L).

To confirm the changes observed in HNF-1α and GATA-4 expression, Western blots were performed with nuclear extracts obtained from isolated enterocytes at different time during postnatal development. A profound increase in HNF-1α protein level was observed during the suckling-Weaning transition, whereas the level of histone deacetylase-2, a protein used as a control, remained relatively unchanged (Fig. 4A). GATA-4 protein level was decreased toward the distal part of the intestine at both days 17 and 22 with no detectable level in the colon (Fig. 4B). Although the level of Cdx2 protein increased toward the distal part of the intestine, both HNF-1α and histone deacetylase-2 proteins remained relatively unchanged (Fig. 4B). Thus, HNF-1α and GATA-4 are subjected to a complex regulation during postnatal development that parallels the restricted induction of SI gene transcription in the differentiated epithelium of the small intestine (5, 6, 31, 32).

HNF-1α, GATA-4, and Cdx2 Interact in Vitro and in Vivo—We next explored whether HNF-1α, GATA-4, and Cdx2 could physically interact. An in vitro GST pulldown assay was performed with the use of recombinant GST-GATA-4 and GST-HNF-1α proteins and in vitro translated [35S]methionine-labeled HNF-1α (H-1α, lane 5), GATA-4 (G4, lanes 6, 8, 9, and 11), and Cdx2 (lanes 2, 3, 4, 7, 8, 10, and 11) proteins. After repeated washing procedures, the labeled proteins were subjected to SDS-PAGE and detected by autoradiography. B, beads; I, 20% input. B, an IP-EMSA was performed with the use of total extracts from HEK293T cotransfected with empty-FLAG, HNF-1α, and GATA-4 expression vectors (IP-F) or with FLAG-Cdx2, HNF-1α, and GATA-4 (IP-F-Cdx2). After the IP, proteins attached to the beads were dissociated by deoxycholate treatment and subjected to an EMSA with the use of SIF1-labeled oligonucleotides (lanes 1 and 2), SIF3-labeled oligonucleotides (lanes 3–5), or GATA-1-globin-labeled oligonucleotides (lanes 6–8). When indicated, specific antibodies (ab) for HNF-1α (a) and GATA-4 (G4) were included in the binding reaction for supershift analysis (arrows). Uncharacterized complexes are indicated by asterisks.

![Figure 4](image4.png)

**Fig. 4.** Western analysis of HNF-1α, GATA-4, and Cdx2 in the mouse intestinal epithelium during the suckling-Weaning transition. **A,** nuclear extracts were prepared from isolated epithelial cells of the jejunum from postnatal (p.n.) days 13 to 24 and analyzed by Western blot with the use of an HNF-1α polyclonal antibody. The blot was stripped and incubated with a histone deacetylase-2 (HDAC-2) polyclonal antibody to monitor protein integrity. **B,** nuclear extracts were prepared from isolated epithelial cells of the jejunum (Je), ileum (Il), and proximal colon (PC) at postnatal days 17 and 22 and analyzed by Western blot with the use of HNF-1α, GATA-4, Cdx2, and histone deacetylase-2 polyclonal antibodies.

![Figure 5](image5.png)

**Fig. 5.** HNF-1α, GATA-4, and Cdx2 physically interact. A, a GST pulldown assay was performed with GST-GATA-4 (gst-G4, left panel), GST-HNF-1α (right panel), or GST (as a control of specificity) attached to Sepharose beads and incubated with in vitro translated [35S]methionine-labeled HNF-1α (H-1α, lane 5), GATA-4 (G4, lanes 6, 8, 9, and 11), and Cdx2 (lanes 2, 3, 4, 7, 8, 10, and 11) proteins. After repeated washing procedures, the labeled proteins were subjected to SDS-PAGE and detected by autoradiography. B, beads; I, 20% input. B, an IP-EMSA was performed with the use of total extracts from HEK293T cotransfected with empty-FLAG, HNF-1α, and GATA-4 expression vectors (IP-F) or with FLAG-Cdx2, HNF-1α, and GATA-4 (IP-F-Cdx2). After the IP, proteins attached to the beads were dissociated by deoxycholate treatment and subjected to an EMSA with the use of SIF1-labeled oligonucleotides (lanes 1 and 2), SIF3-labeled oligonucleotides (lanes 3–5), or GATA-1-globin-labeled oligonucleotides (lanes 6–8). When indicated, specific antibodies (ab) for HNF-1α (a) and GATA-4 (G4) were included in the binding reaction for supershift analysis (arrows). Uncharacterized complexes are indicated by asterisks.
HNF-1α and GATA-4 expression vectors. Immunoprecipitated FLAG-Cdx2 (IP-FLAG-Cdx2) extract interacted with the SIF1 element, whereas the immunoprecipitated FLAG did not produce any specific band (Fig. 5B, lanes 1 and 2). The IP-FLAG-Cdx2 produced specific complexes when incubated with the SIF3- or GATA-labeled oligonucleotides in contrast to the IP-F control extract (Fig. 5B, compare lanes 4 and 7 with lanes 3 and 6). The addition of HNF-1α or GATA-4 polyclonal antibodies resulted in the supershift of SIF3 and GATA upper complexes, respectively, confirming the presence of these proteins in the
IP-FLAG-Cdx2 (Fig. 5B, lanes 5 and 8). These results suggest that native HNF-1α, GATA-4, and Cdx2 proteins coexist in the same complex.

**HNF-1α, GATA-4, and Cdx2 Cooperate to Activate Intestinal Promoter Activity**—To characterize further the functional significance of the protein-protein interaction among HNF-1α, GATA-4, and Cdx2 on SI gene transcription, we performed cotransfection experiments in Caco-2 cells with the use of the SI promoter linked to a luciferase gene reporter. Similar constructs harboring mutations within HNF-1, GATA, and Cdx interacting sites were also used to monitor the specificity of these interactions (Fig. 6A). These mutations have been characterized previously by EMSA (10, 37). Cotransfection of either Cdx2 or HNF-1α expression vector with the SI promoter construct increased the transcriptional activity, whereas cotransfection of the GATA-4 expression vector alone did not significantly influence SI activity in Caco-2 cells (Fig. 6B). Although some minor synergy in the transcriptional activation of SI was observed when both Cdx2 and HNF-1α were combined, the addition of GATA-4 with both Cdx2 and HNF-1α enhanced the synergistic activation of the SI promoter (Fig. 6B). This effect was observed only when Cdx2 and HNF-1α were simultaneously cotransfected with GATA-4 (Fig. 6B). Similar results were observed when an IPAL promoter-luciferase construct was used in the cotransfection assay (Fig. 6B), suggesting that the interaction among HNF-1α, GATA-4, and Cdx2 could be important for the regulation of other intestinal genes. A Western blot was performed in parallel to confirm the production of HNF-1α, GATA-4, and Cdx2 proteins in these cotransfection experiments (Fig. 6C). Individual mutations within the HNF-1 and Cdx sites of the SI promoter resulted in a reduction of the synergistic effect of HNF-1α, GATA-4, and Cdx2 on SI gene promoter activity, whereas a mutation in the GATA site did not affect the combined effect of these factors on SI transcriptional activity (Fig. 6D). A Western blot confirmed that similar levels of HNF-1α, GATA-4, and Cdx2 proteins were produced in these cotransfection experiments (Fig. 6E). These results confirmed that GATA-4 synergies with HNF-1α and Cdx2 in the transcriptional activation of the SI promoter and a functional GATA-interacting site is not required for this effect.

**DISCUSSION**

The understanding of molecular mechanisms that govern tissue-specific gene expression often lead to the identification of transcription factors responsible for tissue specialization and maturation. In the past and herein, we used the SI gene as a model to study the molecular basis of the establishment and maintenance of the intestinal phenotype. A short evolutionarily conserved SI promoter contains cis-acting elements that interact with HNF-1α, GATA-4, and Cdx proteins. Interestingly, similar elements are present in a number of intestinal specific gene promoters such as IPAL (29), lactase-phlorizin hydrolase (21, 38, 39), and intestinal fatty acid-binding protein (24, 40). We have recently described the importance of the HNF-1 and GATA elements in the regulation of SI promoter activity in vivo (18, 20). In the current study, we showed that the Cdx element is essential for SI gene transcription. Our findings also indicated that HNF-1α protein expression was induced in the villous epithelium during the suckling-weaning transition. In addition, GATA-4 protein was restricted to the villous epithelium with a decreasing gradient of expression along the horizontal axis of the intestine. We demonstrate that HNF-1α, GATA-4, and Cdx2 interact and cooperate for the transcriptional activation of intestine-specific genes. Our results imply that the Cdx element is required for SI gene transcription but that combinatorial interactions of HNF-1α, GATA-4, and Cdx2 are important for the temporal and spatial regulation of SI gene expression during postnatal development.

The HNF-1α protein is detected in only a few epithelial cells in suckling animals to become progressively expressed in differentiated enterocytes during the suckling-weaning transition. In adult mice, HNF-1α mRNA has been found to be highest in crypts of the small and large intestine with a decreasing gradient along the vertical axis (19). Our analysis shows that HNF-1α protein is detected abundantly in villus enterocytes with only minor expression in the crypt compartment. The molecular network involved in the regulation of HNF-1α has been characterized partly in embryoid bodies (41) but remains largely unknown in the intestinal epithelium context. GATA-4 protein expression is also restricted to villous epithelial cells but is not expressed in the most distal part of the intestine. GATA-4 transcripts were originally reported to be present in the mouse small intestinal villus region and undetectable in the colon (23). Other studies have reported that the GATA-4 mRNA was localized in the entire tract of the intestinal epithelium late in mouse embryonic development (42) and up-regulated along the crypt-villus axis in chicken (24). Our findings demonstrate that the GATA-4 protein is restricted to the small intestinal villus region and absent in the colon, a pattern that is established early during embryonic development. The molecular mechanisms that control GATA-4 expression are still to be determined. Our findings coupled to others suggest that regulation of HNF-1α and GATA-4 relies on transcriptional and post-transcriptional mechanisms that promote the regionalization of gene expression along both the horizontal and vertical axes during intestinal development.

The overall distribution of HNF-1α, GATA-4, and Cdx2 proteins during the suckling-weaning transition corresponds well with the induction of SI (6) and IPAL (43) mRNA expression. Nevertheless, it is likely that post-translational modifications may play a role in the functional interaction of these factors to regulate intestinal gene transcription. We have reported previously that phosphorylation of the serine 60 residue within the Cdx2 protein which occurs in the crypt compartment reduces its trans-activation capacity (15). The phosphorylation of GATA-4 by certain activated kinase pathways influences both binding and trans-activation potency (44, 45). Two different GATA family members (GATA-1 and GATA-3) are direct targets of CREB-binding protein histone acetyltransferase activity, and the acetylation of family conserved GATA-specific domains results in the modification of their activation capacity (46–48). In addition, other transcription factors and cofactors are likely to be involved in the modulation of SI gene expression. For example, Cux/CDP interacts with and represses SI promoter activity via an element that encloses the GATA site (20). Furthermore, Cdx2 and GATA-4 interact with the cofactor p300 (49, 50), whereas HNF-1α interacts with both CREB-binding protein and its associated factor PCAF (51). Whether these post-translational and physical interactions occur along the vertical and horizontal axes of the intestine during development remains to be determined.

The Cdx2 and HNF-1α proteins can interact via their respective homeodomain region in vitro (52). Our results suggest that Cdx2, HNF-1α, and GATA-4 all interact with each other and are part of a same complex. However, detailed mechanisms and the nature of the domains involved for the formation of a stable complex with transcriptionally active intestinal genes in vivo will need further studies. It has been suggested that GATA proteins can influence the activity of certain gene promoters without physically interacting with DNA but through physical interactions between other proteins binding to regulatory ele-

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2 F. Boudreau and G. P. Swain, unpublished observations.
ments of the promoter (53). GATA-5 and HNF-1α cooperatively activate the lactase gene promoter, an effect that is independent of an intact GATA interacting site (21). Our results suggest that the transcriptional activation of the SI promoter by GATA-4 does not require an intact GATA site but depends on Cdx2 and HNF-1α. Therefore, it is possible that other family members such as GATA-5 and GATA-6 could influence SI promoter activity via similar indirect interactions.

The importance of Cdx2 in the maintenance of the intestinal phenotype has been demonstrated in knockout experiments (54, 55). Ectopic expression of Cdx2 within the stomach epithelium results in the formation of intestinal metaplasia (56). The GATA-4 protein plays a role in the specification of the mouse gastric epithelium as demonstrated with the use of chimeric mice (57) and stimulates the differentiation of embryonic cells into extraembryonic endoderm (58). The possible role of HNF-1α in the formation and maintenance of the gastrointestinal tract has not been yet characterized carefully (59, 60). The future challenge will be to investigate the combinatorial roles of HNF-1α, GATA-4, and Cdx2 in the morphogenesis and differentiation of the intestinal epithelium during development.

Acknowledgments—We thank Yi Zhu, Jennifer Paolella, Sandy Mancano, and João Pedro Teixeira for excellent technical assistance. We also thank Dr. M. S. Parmacek for providing the GATA-4 expression vector.

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