Sucrase-isomaltase Gene Transcription Requires the Hepatocyte Nuclear Factor-1 (HNF-1) Regulatory Element and Is Regulated by the Ratio of HNF-1α to HNF-1β*

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The mouse sucrase-isomaltase (SI) gene is an enterocyte-specific gene expressed in a complex developmental pattern. We previously reported that a short, evolutionarily conserved gene promoter regulates developmental expression of SI in mouse small intestine. Herein, we investigated the role of a hepatocyte nuclear factor-1 (HNF-1) cis-acting element to regulate SI gene expression in vivo. Transgenic SI gene constructs with a mutated HNF-1 element (SIF3) revealed a strong reduction in promoter activity in comparison with a wild-type construct in mice and during Caco-2 cell differentiation. Nuclear proteins isolated from enterocytes showed increased binding of the HNF-1α complex with a concomitant decrease in the HNF-1β-containing complex to the SIF3 element both during the suckling-weaning developmental transition and Caco-2 cell differentiation. These changes coincided with a strong induction of SI gene transcription. In transfection experiments, HNF-1α activated the SI promoter via the SIF3 element, and co-expression of HNF-1β impaired this transcriptional activation. These findings demonstrate the essential role of the HNF-1 regulatory element to support SI gene transcription in vivo and suggest that the ratio of HNF-1α to HNF-1β plays a role in the transcriptional activity of this gene during intestinal development.

Sucrase-isomaltase (SI) is an intestine-specific gene that is expressed in complex patterns in epithelial cells during development along the cephalocaudal and the crypt-villus axis of the intestine (1–3). In the mouse intestine, SI is initially expressed at low levels late in fetal development when the stratified endodermal epithelium transforms into a columnar epithelium with nascent villi (3). This low level of SI expression is maintained through birth and the first two postnatal weeks of life (3). Between days 16 and 17, there is a dramatic induction in SI expression that occurs in concert with other changes in gene expression that results in the phenotype of the adult intestine (3). During this transition and throughout adult life, there is a prominent induction of SI mRNA in enterocytes located at the crypt-villus junction. SI mRNA is abundant in enterocytes from the base of villi to the midvillus toward the tip, resulting in low levels in villus tip cells (4, 5). This pattern of expression mirrors critical developmental and differentiation transitions in the intestine. Therefore, SI has served as a useful model to investigate the molecular mechanisms that orchestrate intestinal developmental transitions.

We have mapped regions of the SI gene that are important to recapitulate its complex expression during mouse intestinal development using transgenic mice (1–3). This analysis shows that the crucial regulatory elements that direct patterns of enterocyte expression are located between nucleotides −201 and +54 of the SI promoter. This short, evolutionarily conserved mouse SI gene promoter directs transcription to enterocytes in developmental and differentiation-dependent patterns (3). Since these transgenic constructs are able to recapitulate the expression profile of the endogenous gene during development, we conclude that increased expression of the SI gene at the suckling-weaning transition is due predominantly to induction of transcription. Moreover, the crucial regulatory elements that direct patterns of enterocyte expression are located between nucleotides −201 and +54 of the SI promoter.

Further studies of the human SI promoter in cell lines have identified three major positive regulatory elements, sucrase-isomaltase footprint 1 (SIF1), SIF2, and SIF3 (6, 7). Caudal-related homeodomain proteins (CdX1 and CdX2) interact with the SIF1 element and induce gene transcription in vitro (8, 9). The SIF2 and SIF3 elements of the human SI promoter interact with HNF-1 proteins to regulate transcription (10). It is currently unclear whether these proteins interact with the SI promoter in vivo to regulate gene transcription during intestinal development.

In this study, we investigate the role of HNF-1 proteins in the transcriptional regulation of the SI promoter during postnatal intestinal development. HNF-1α and HNF-1β are transcription factors distantly related to homeodomain proteins that bind to DNA as either homodimers or heterodimers (11). They are expressed in a variety of tissues including liver, kidney, intestine, stomach, and pancreas and have been implicated in the regulation of multiple genes (12–17). In this report, we demonstrate that mutagenesis of the SIF3 element strongly impairs the activity of SI promoter in transgenic mice and Caco-2 cells. Using intact nuclear proteins from neonatal mouse intestinal epithelial cells and Caco-2 cells, we show that the pattern of protein binding to the SIF3 element changes at a period when SI expression is dramatically increased. We find that an increase in the ratio of HNF-1β to HNF-1α reduces SI promoter activity. Taken together, these data confirm an es-

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The abbreviations used are: SI, sucrase-isomaltase; mSI, mouse SI; SIF, sucrase-isomaltase footprint; HNF-1, hepatocyte nuclear factor-1; hHNF-1, human HNF-1; hGH, human growth hormone; PCR, polymerase chain reaction; bp, base pairs; CMF, calcium/magnesium-free Hanks’ balanced salts; EMSA, electrophoretic mobility shift assay.
Siential role for the HNF-1 element to regulate expression of the SI gene during intestinal development and enterocyte differentiation.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction and Mutagenesis**—The −201 to +54 mSI-hGH construct has been described elsewhere (3). Point mutagenesis of the −201 to +54 mSI-hGH plasmid was performed using the Transformer site-directed mutagenesis kit (CLONTECH). The following oligonucleotide was designed to create point mutations (underlined) within the SIF3 element: SIF3m, 5′-GGTTGAATCTCAGTCGACC-3′ and mSIF, 5′-GTTYGAAATACACAGGTATTAAGTAACT-3′. Correct orientation of the −342 to −201 integrated fragment as well as the presence of the SIF3 mutation in the −201 to +54 mSI-hGH construct was confirmed by sequence analysis. Portions of the human and mouse ribosomal protein 36B4 mRNA and the human HNF-1α mRNA were amplified by reverse transcriptase-PCR. Reverse transcription reactions were performed as described previously (3) with the use of either RNA extracted from Caco-2 cells or mouse small intestine. For PCR, the following paired primers were used for amplification: for h36B4, h36B4up (5′-ATGTGAGGTCACTGTGCCAG-3′) and h36B4down (5′-GGAATCTCAGTCGACC-3′); and for hHNF-1α, h1F1oup (5′-GGAATCTCAGTCGACC-3′) and hHNF1edown (5′-GGAATCTCAGTCGACC-3′). PCR amplification was carried out for 35 cycles with denaturation at 95 °C, annealing of the primers at 55 °C, and extension at 72 °C, each for 1 min. A 113-base pair fragment extending from positions 431 to 544 of h36B4, a 420-base pair fragment from positions 428 to 848 of m36B4, and a 643-base pair fragment from position 2465 to 3108 of hHNF-1α cDNA were subcloned into the pGEM-T easy vector (Promega, Madison, WI) according to the manufacturer’s recommendations. The integrity of subcloned PCR products was confirmed by sequencing analysis. The 132- bp EcoRI-Smal m36B4 fragment was further subcloned in Bluescript KS vector (Stratagene, La Jolla, CA) to create the KS-m36B4 plasmid.

**Transgenic Mice**—The promoter/reporter −8.5 to +54 SIF3m mSI-hGH construct was released by digestion with SalI and SphI and purified. Transgenic mice were produced by the Transgenic Core Facility at the University of Pennsylvania. The DNA construct was injected into the male pronucleus of fertilized eggs and implanted into pseudo-pregnant females using standard methods. DNA from tail tip fragments of resulting mice was extracted using the QIAamp Tissue kit (Qiagen Inc., Valencia, CA). The presence of the transgene in mouse genomic DNA was determined by PCR and Southern analysis as described previously (2, 3). Transgene founders of the B6SJFl strain (Jackson Laboratory, Bar Harbor, ME) were bred with normal CD1 mice (Charles River), and offspring were analyzed by PCR to determine the genotype by PCR.

**Cell Culture**—A Caco-2 cell line was obtained from the American Tissue Culture Collection (Manassas, VA). The Caco-2–15 cell line was kindly provided by Dr. J.-F. Beaulieu (Université de Sherbrooke, Québec). Cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 4.5 g/liter D-glucose, 25 mM HEPES, 10% fetal calf serum, 10 g/ml of streptomycin in 5% CO2.

**Isolation of Nuclear Proteins from Intestinal Epithelium**—Nuclear proteins was isolated from neonatal mice by a modification of a method previously used by our laboratory for adult mouse intestine (6). Briefly, mice were anesthetized with sodium pentobarbital (0.15 ml for adult, 0.05 ml for neonatal mice), and the intestine was exposed through a midline incision. The distal small intestine was rinsed with 10 ml of 1× calcium/magnesium-free Hank’s balanced salt solution (CMF). The thoracic cavity was opened, and the left ventricle was perfused through a needle with 1× CMF for 3 min with a hydrostatic head of 5 feet. Perfusion fluid was allowed to exit through an incision in the right atrium. Adequacy of the perfusion was determined by homogenous blanching of the intestine and liver. The section of bowel that was initially washed with 10 ml of 1× CMF buffer containing 2 μg/ml leupeptin, 2 μg/ml aprotinin, 2 μg/ml bestatin, 2 μg/ml pepstatin A, and 0.2 mM phenylmethylsulfonyl fluoride to collect epithelial cells. The cells were washed twice with 1× CMF containing proteinase inhibitors. Nuclear proteins were then isolated from the epithelial cell suspension using the same method as previously described for cells in culture. Following each washing, the isolated epithelium was stained with trypan blue to examine cell integrity. Cells isolated with greater than 5% trypan blue-positive cells, indicating disrupted membranes, were discarded.

**Protein Analysis**—Nuclear proteins were isolated from Caco-2 cells or intestinal epithelial cells (see above), and EMSA was performed exactly as described previously (8). HNF-1α affinity-purified goat polyclonal antibody against the carboxy terminal of human HNF-1α (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and HNF-1β affinity-purified rabbit polyclonal antibody raised against a peptide mapping to the N terminus of human HNF-1β were used for supershift experiments. For Western blot analysis, 20 μg of total protein extract was analyzed by a 4–12% Bis-Tris NuPAGE (Invitrogen, Carlsbad, CA) electrophoresis and transferred to an Immobilon-P membrane. The membrane was blocked with 10% nonfat dry milk in phosphate-buffered saline and 0.1% Tween for overnight. The membrane was then incubated for 3 h with the primary antibody. The following antibodies were used: HNF-1 affinity-purified rabbit polyclonal antibody raised against a peptide mapping to amino acids 80–284 of human HNF-1α that reacts with HNF-1α and HNF-1β proteins (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and rabbit polyclonal antibody raised against a peptide mapping to the N terminus of murine Cdx2.2 The membrane was washed two times in phosphate-buffered saline with 0.1% Tween and incubated with the secondary antibody anti-rabbit/ horseradish peroxidase (Amersham Pharmacia Biotech) for 45 min at room temperature. The membrane was then washed three times and developed with the ECL-Plus Western blotting kit (Amersham Pharmacia Biotech).

For immunohistochemistry, tissues were fixed in 10% buffered formalin (Fisher) and embedded in paraffin in Swiss roll orientation such that the entire length of the intestinal tract could be identified on a single section. Tissue sections were stained for hGH protein and counterstained with eosin as described previously (1–3). RNase Protection Assays—RNA Analysis—RNA was extracted from multiple tissues using a Trizol–Life Technologies, Inc.) Ribonuclease (RNase) protection assays were performed using the RPA II kit (Ambion, Austin, TX) according to the manufacturer’s recommendations. Riboprobes for the detection of human SI and hGH mRNA were prepared as previously described (1). The riboprobe for the detection of hHNF-1α was prepared by digesting pGEM-hHNF1-α with XhoI, and transcripts were synthesized using SP6 RNA polymerase to yield a probe that protects a 326-bp region located in the 3′-noncoding region of the hHNF-1α cDNA. As a control for total RNA integrity, human and mouse riboprobes for the detection of the ribosomal protein 36B4 mRNA (18) were synthesized. h36B4 riboprobe was prepared by digesting pGEM-h36B4 with SacII and with SP6 RNA polymerase to synthesize using SP6 RNA polymerase to yield a probe that protected 132 nt of m36B4 riboprobe was prepared by digesting KS-m36B4 with HindIII and transcribed with T7 RNA polymerase to yield a probe that protected 132 nt. Northern blot was performed as described elsewhere (19). Not1-XhoI mouse HNF-1β cDNA fragment digested from pBJ5-HNF-1β plasmid (16) was used to detect the human HNF-1β mRNA, since comparison of cDNA nucleotide sequences showed more than 91% homology. h36B4 was used as a control for RNA integrity.

**Transient Transfections**—Transient transfections were performed using LipofectAMINE (Life Technologies) according to the manufacturer’s recommendations. Cells at 50–60% confluence were incubated with 1.5 μg of total DNA and 2.5 μl of LipofectAMINE/ml of OPTI-MEM for 5 h. The medium was then changed to Dulbecco’s modified Eagle’s medium complete medium containing 10% fetal bovine serum. For transient transfections, luciferase activity was determined 48 h after the transfection using the luciferase assay kit (Promega Biotech, Madison, WI). Each experiment was repeated three times in triplicate. pCMV-β-galactosidase expression vector was co-transfected in each experiment as a measure of transfection efficiency, and the results were reported as light units per unit of β-galactosidase. For stable transfections, Caco-215 cells were transplanted 24 h following transfection and maintained in selection medium containing 1 mg/ml G418.

2 F. Boudreau and P. G. Traber, unpublished results.

3 E. H. M. Rings and P. G. Traber, unpublished results.
RESULTS

Mutagenesis of the SIF3 Element Impairs SI Promoter Activity in Transgenic Mice—To evaluate the functional role of the SIF3 element in the regulation of the SI gene in the intact mouse intestinal epithelium, we designed transgenic constructs harboring point mutations within this element that linked nucleotides −8.5 to +54 of the mSI gene to the hGH reporter gene (Fig. 1A). The wild-type construct supports hGH expression in enterocytes of transgenic mice and has been shown to direct copy number-dependent and insertion site-independent transgene expression in multiple transgenic lines (2). The SIF3m mutation has been previously shown to abolish in vitro interaction with HNF-1 proteins (10).

Four founders (numbers 17, 28, 29, and 41) derived from the construct −8.5 to +54 SIF3m mSI-hGH were found to have 1, 31, 7, and 10 copies of the transgene integrated into their genome, respectively (data not shown). The effect of the SIF3 mutation on hGH expression in these transgenic lines was compared with the previously characterized F0(17)F1(32) transgenic line that contained five copies of the wild-type transgene construct (2). Total RNA isolated from different portions of the intestine was analyzed by a RNase protection assay. Each RNA sample was incubated with probes for hGH and an internal control (36B4) in the same hybridization solution in order to correlate hGH mRNA levels among these different lines. The wild-type F0(17)F1(32) transgene line showed high level expression of hGH in the small intestine (Fig. 1B), as previously reported (2). In contrast, the expression of hGH mRNA in the transgenic lines carrying the mutant construct was reduced by more than 95% in the duodenum and proximal and distal jejunum of founders 17, 29, and 41 and by 85% in founder 28 as compared with the expression found in the wild-type transgene line (Fig. 1, B–D). Expression levels were similarly decreased in the ileum, although the pattern was somewhat more variable (Fig. 1D). No hGH expression was detected in transgenic lines harboring the same SIF3 mutation within a shorter −201 to +54 mSI/hGH transgene as compared with the wild type short transgene (data not shown).

To verify the cellular distribution of hGH expression in the −8.5 to +54 SIF3m mSI-hGH transgenic lines, immunodetection was performed. Intestinal sections obtained from offspring of transgenic mice containing the wild-type transgene construct showed high level expression of hGH in differentiated enterocytes of the small intestine (Fig. 2A). In contrast, weak expression was detected in enterocytes randomly distributed along the proximal jejunum villi from offspring of founders 28 and 41, as illustrated in Fig. 2, B and C. Expression was restricted to the proximal jejunum with no detectable hGH protein throughout the entire small bowel as opposed to the wild-type transgenic control (data not shown) (2). In summary, the data obtained from these transgenic constructs demonstrate a crucial role for the SIF3 element to regulate SI promoter activity in the small intestinal epithelium.
The SIF3 Element Is Important in the Regulation of SI Promoter Activity in Caco-2 Cells—The molecular mechanisms involved in the SIF3-dependent regulation of SI gene expression was further examined in the Caco-2 cell line, which has been extensively used as a model of enterocytic differentiation (20–22). We used the Caco-2/15 clone derived from the parent Caco-2 cell line that spontaneously differentiates in postconfluence with concomitant high induction of SI expression (23). We investigated the role of the SIF3 element to regulate SI promoter activity during Caco-2 cell differentiation. The constructs −8.5 to +54 mSI-hGH and −8.5 to +54 SIF3m mSI-hGH were co-transfected with a neomycin resistance expression vector (pRC/CMV) to stably transfect Caco-2/15 cells. As a control, Caco-2/15 cells were co-transfected with the empty hGH reporter and pRC/CMV. Because of the heterogeneous properties of Caco-2 cell lines with regards to SI expression (24), the entire neomycin-resistant population was used for further analysis. Expression of human SI, hGH, and h36B4 was determined in RNA samples isolated at different times of Caco2 cell confluence. The expression level of hGH mRNA was reported to h36B4 signal and represented graphically (mean ± S.D.).

Caco-2 Cell Differentiation—We next examined the pattern of nuclear protein interaction with the SIF3 element during mouse intestinal development. To obtain high quality nondegraded protein, we performed a whole-body perfusion to isolate enterocytes and extract nuclear proteins from mouse intestines at different time points throughout the suckling-weaning transition. To ensure that our method isolated both crypt- and villus-associated cells, we isolated mRNA from the epithelial cells, and the expression of specific intestinal epithelial genes was examined by Northern analysis. We found that both SI (villus marker) and cryptdin (crypt marker) mRNAs were found in cell extracts (data not shown). Therefore, both crypt and villus cells were represented in the epithelial cell preparations. EMSA was then performed using a labeled SIF3 DNA probe, and equal amounts of nuclear extracts from intestinal epithelial cell pools were collected at different time before and after weaning.

The SIF3 element produced two specific complexes that were competed by the addition of a 100-fold excess of unlabeled SIF3 oligonucleotides (Fig. 4A). Although both complexes were detected at each time points during intestinal development, the relative intensity of the two complexes varied. The lower complex (labeled B in Fig. 4A) was more abundant than the upper complex (A) at postnatal day 13, a time at which SI mRNA is just detectable (3). By postnatal day 19, the ratio of A to B complexes shifted, resulting in more abundant complex A relative to B. Importantly, this period of time coincides with...
dramatic induction of SI mRNA levels in the small bowel (3). The change in ratio of these complexes was further amplified in adult mice (Fig. 4A). Analysis of nuclear extracts binding to the mouse SIF3 element showed no specific DNA-protein interaction during mouse intestinal development. A similar analysis was then performed using the labeled SIF3 DNA probe and an equal amount of nuclear extracts prepared from preconfluent and postconfluent Caco-2 cells. The SIF3 element produced two protein-DNA complexes (A and B in Fig. 4B), both of which were eliminated by competition with a 10-fold excess of unlabeled SIF3 oligonucleotides. The intensity of complex A increased at postconfluent day 7 with a simultaneous decrease in intensity of complex B, a pattern that was maintained at postconfluent day 14 (Fig. 4B). The addition of affinity-purified polyclonal HNF-1α antibodies to the binding reaction super-shifted both complex A and complex B, while HNF-1β antibodies supershifted only complex B, confirming that complex A is composed of HNF-1α homodimers and complex B is composed of HNF-1α/HNF-1β heterodimers (Fig. 4C). These observations suggested that HNF-1 isoforms are differentially regulated during the suckling-weaning transition and Caco-2 cell differentiation. Therefore, we examined the expression of HNF-1α and HNF-1β mRNA at different times during Caco-2 cell growth and differentiation. RNase protection assay performed with specific HNF-1α and h36B4 probes showed no significant change in HNF-1α mRNA levels in comparison with 36B4 mRNA levels before or after Caco-2 cell confluence (Fig. 5A). However, HNF-1β mRNA levels were reduced more than 3-fold at postconfluence as determined by Northern analysis (Fig. 5B). Immunoblots were performed to test whether changes in protein expression correlated with RNA expression of these isoforms during Caco-2 cell differentiation. No major change in HNF-1α protein level was observed before or after Caco-2 cell differentiation (Fig. 5C). However, HNF-1β protein level was decreased more than 2.5-fold at postconfluence (Fig. 5C). Interestingly, CDX2 protein content was not significantly modified during Caco-2 cell differentiation (Fig. 5C).

The Ratio of HNF-1β to HNF-1α Regulates SI Promoter Activity in Caco-2 Cells—We then evaluated whether changes in the relative levels of HNF-1 isoforms have a functional effect on the regulation of SI gene transcription. Co-transfection of an HNF-1α expression vector with the −201 to +54 SI promoter construct produced an increase in transcriptional activity of the SI promoter construct in Caco-2 cells (Fig. 6A). In contrast, expression of HNF-1β led to minimal activation of the construct (Fig. 6A). A point mutation in the SIF3 element that abolishes binding of HNF-1 proteins to the site (10) eliminated the positive effect of HNF-1α on the SI promoter (Fig. 6A). We then tested whether HNF-1β could counter the positive effect of HNF-1α. High amounts of HNF-1β expression vector resulted in decreased activation of the SI promoter construct when a constant amount of HNF-1α was maintained (Fig. 6B). Nuclear complexes binding to the SIF3 element reflected the increased ratio of HNF-1β to HNF-1α in these co-transfection experiments where the HNF-1α homodimer complex decreased with associated augmentation in both HNF-1α/HNF-1β heterodimer and HNF-1β homodimer complexes (Fig. 6C). Both HNF-1α homodimer and heterodimer complexes were detected in the condition where HNF-1β was co-transfected alone (Fig. 6C, third lane), since Caco2 cells express both endogenous HNF-1α and HNF-1β proteins (Fig. 5C). These results confirm that the ratio of HNF-1 proteins binding to the SIF3 element is critical in the regulation of the SI promoter in intestinal epithelial cells.

DISCUSSION

The epithelium lining the intestine undergoes a complex series of developmental transitions that result in the functional
adult intestinal mucosa. Examination of the mechanisms that
direct expression of developmentally regulated genes provides
a better understanding of these developmental processes. In
postnatal development, the suckling-weaning transition is
marked by profound changes in intestinal gene expression and
epithelial cell function. During this time, there is a marked
induction of SI expression, which parallels other important
developmental transitions in the intestine and therefore has
served as a model for elucidating molecular regulation of de-
velopmental events (25). Our results show that one cis-acting
DNA regulatory element is critically important to support SI
gene transcription during postnatal development and intesti-
 nal epithelial cell differentiation. Moreover, the activity of this
regulatory site is dependent on the relative amounts of two
related transcription factors, HNF-1\(_\alpha\) and HNF-1\(_\beta\).

Previous work from our laboratory has suggested a role for
HNF-1 transcription factors to regulate human SI promoter
activity (10). The use of human nuclear extracts isolated from
intestinal cell lines has led to the identification of two different
elements, SIF2 and SIF3, both interacting with HNF-1 pro-
teins (6, 10). Mutation of the SIF3 element strongly reduced
mouse SI promoter activity in transgenic mice and Caco-2 cells.
HNF-1\(_\alpha\)-dependent induction of SI promoter activity was
strongly reduced when the SIF3 element was disrupted in co-transfection experiments. The current studies show that the
SIF2 element is not functionally important for the mouse SI
promoter. No specific interaction was found with mouse intesti-
 nal nuclear extracts and the mouse SIF2 element, most likely
because the second half of the mouse SIF2 element (AACATT)
differs from the HNF-1 consensus site (ATTAAC). Moreover,
there is one nucleotide difference between the human and
mouse genes in the first half-region of the element. Therefore,
we conclude that the SIF3 regulatory region solely interacts
with HNF1 proteins to regulate mouse SI gene expression at
the suckling-weaning transition.

**FIG. 5.** Expression of HNF-1\(_\alpha\) and HNF-1\(_\beta\) during Caco-2 cell
differentiation. A, total RNA was extracted at different days of Caco2
cell confluence (PC, preconfluent; C, confluent; +7, 7 days after conflu-
ence; +14, 14 days after confluence). RPA was performed using
hHNF1\(_\alpha\) and h36B4 riboprobes. B, Northern analysis was performed
using sequentially a mouse HNF1\(_\beta\) \(^{32}\)P-labeled cDNA probe and a
h36B4 \(^{32}\)P-labeled cDNA probe as a control for total RNA loading. C,
Western blot analysis was performed using HNF-1 and Cdx2 polyclonal
antibodies on similar amounts of total protein extracted at different
days of Caco2 cell confluence.

**FIG. 6.** Effect of HNF1\(_\alpha\) and HNF1\(_\beta\) on SI transcriptional ac-
tivity in Caco-2 cells. A and B, Caco-2/15 cells were transfected using
LipofetAMINE (Life Technologies) with 300 ng of either −201 to +54
mSI-pGL2basic (WT) or −201 to +54 SIF3m mSI-pGL2basic (SIF3m)
reporter vectors, 100 ng of pCMV-\(\beta\)-galactactosidase, and various
amounts of HNF-1\(_\alpha\) or HNF-1\(_\beta\) expression vectors or both, as indicated.
The pRC/CMV plasmid was used as an empty control vector to calibrate
the various amounts of expression vectors used in each condition. Re-
sults obtained in triplicate were reported as -fold difference (mean ±
S.D.) from transfection with the reporter construct alone and are re-
presentative of three independent experiments. C, EMSA of the SIF3
element was performed with nuclear extracts from Caco-2/15 cells co-
transfected as described for A and B.
The increase of the HNF-1α homodimer complex that binds to the SIF3 regulatory element correlates very well with the induction of SI gene expression both at the suckling-weaning transition (3) and during Caco-2 cell differentiation. Changes in the binding activity of HNF-1α during Caco-2 cell differentiation have also been observed for specific elements found in the human α1-antitrypsin promoter (26). In this study, Northern analysis showed an increase in the HNF-1α mRNA level during Caco-2 cell differentiation (26). We were unable to detect significant change in HNF-1α mRNA and protein levels with the use of two different Caco-2 cell lines. Differences in the nature of the probe and methods used to detect the messenger RNA may explain this discrepancy. The mechanisms involved in the regulation of HNF-1 dimerization and interaction with the chromatin in intestinal epithelial cells must be complex and will require additional studies. One explanation for the increase in HNF-1α homodimers without any important change in HNF-1α protein content is that the decrease in the ratio of HNF-1β to HNF-1α proteins would lead to a redistribution in the composition of HNF1 complexes that interact with the SIF3 element of the SI promoter.

There are major differences in the transactivation domains of HNF-1 proteins (16). HNF-1β has a positive transactivation effect on some binding sites such as those in albumin and α-fetoprotein (27) or more commonly no functional effect at all, such as in the ß-fibrinogen promoter (16) and the CYP2E1 promoter (28). It has been demonstrated that the relative abundance of both HNF-1 proteins can affect vitamin-D-binding protein gene transcription (29). The inhibitory effect of HNF-1β on HNF-1α has been suggested to be dependent on several DNA binding sites where HNF-1β acts as a trans-dominant inhibitor of HNF1-mediated enhancer activity (29). The mechanism by which HNF-1β reduces HNF-1α transactivation of the mouse SI promoter may be more complex, since a single DNA interacting site is involved. The transactivation properties of HNF-1α have been shown to rely on physical interactions with multiple coactivator proteins such as CREB-binding protein and CBP-associated factor (30). Interestingly, the region of HNF-1α necessary to interact with the CBP-associated factor protein is absent in the HNF-1β protein (30).

Changes in the binding of HNF-1 complexes cannot fully explain the specificity of SI expression in the mature enterocyte, since HNF-1α is more abundant than HNF-1β in tissues where the SI gene is not expressed (16). Furthermore, SI promoter activity is equally detected in the mutant SIF3 and the wild-type mSI reporter constructs at the onset of Caco-2 cell differentiation when SI mRNA expression becomes detectable (Fig. 6). The SIF1 DNA regulatory element is crucial for regulation of the SI promoter in cell lines (6, 7). The element contains two binding sites for Cdx proteins, which are able to activate SI promoter transcription via binding to this site. The Cdx regulatory element is likely to be involved in the specificity of SI gene expression in intestinal epithelial cells, because Cdx2 homeodomain protein regulates both proliferation and differentiation of intestinal epithelial cells (31) and is restricted to the intestinal epithelium during mouse postnatal development (32). A close interaction between Cdx2 and HNF-1 proteins represents an interesting hypothesis for the spatial and time-dependent regulation of SI gene expression during postnatal development. Such an interaction has been suggested to promote lactase transcriptional activity as demonstrated by in vitro studies (33).

In summary, we have showed that the SIF3 cis-acting element is crucial to positively regulate an intestinal specific gene promoter in vitro. We propose that the SIF3 element maintains a pivotal role in the control of SI promoter activity where binding of a predominant HNF-1α homodimer complex promotes the interaction of intestine-specific transcription factors and co-factors with the promoter. Future studies on the in vivo interaction of HNF-1, Cdx2, and co-factors during postnatal development will provide insights to elucidate the molecular mechanisms involved in the regulation of intestine-specific gene expression.

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