Physical Interaction between GATA-5 and Hepatocyte Nuclear Factor-1α Results in Synergistic Activation of the Human Lactase-Phlorizin Hydrolase Promoter

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GATA-4, -5, and -6 zinc finger and hepatocyte nuclear factor-1α (HNF-1α) homeodomain transcription factors are expressed in the intestinal epithelium and synergistically activate the promoter of intestinal genes. Here, we demonstrate that GATA-5 and HNF-1α physically associate both in vivo and in vitro and that this interaction is necessary for cooperative activation of the lactase-phlorizin hydrolase promoter. Furthermore, physical association is mediated by the C-terminal zinc finger of GATA factors and the homeodomain of HNF-1α. Deletion of HNF-1α activation domains or interruption of HNF-1-binding sites in the lactase-phlorizin hydrolase promoter resulted in a complete loss of cooperativity, whereas deletion of GATA-5 activation domains or interruption of GATA-binding sites resulted in a reduction, but not an elimination, of cooperativity. We hypothesize that GATA/HNF-1α cooperativity is mediated by HNF-1α through its activation domains, which are oriented for high levels of activation through binding to DNA and physical association with GATA factors. These data suggest a paradigm whereby intestine-specific gene expression is regulated by unique interactions among tissue-restricted transcription factors coexpressed in the intestine. Parallel mechanisms in other tissues as well as in Drosophila suggest that zinc finger/homeodomain interactions are an efficient pathway of cooperative activation of gene transcription that has been conserved throughout evolution.

The intestinal epithelium is a dynamic structure that undergoes a highly regulated process of cell division, migration, cell fate determination, and differentiation (1–3). During intestinal development, interactions between visceral endoderm and mesoderm at E81 in mice result in the formation of a primitive foregut that rapidly undergoes cytodifferentiation, so that by E19, an epithelial monolayer overlies nascent villi. During the first 2 weeks of postnatal life, a proliferating compartment develops into the crypts of Lieberkühn. Stem cells located near the base of crypts rapidly divide and give rise to four terminally differentiated cell types, which migrate both basally and apically. Cells migrating to the base of crypts become Paneth’s cells, whereas those migrating up the crypt toward villi become absorptive enterocytes, goblet cells, and enteroendocrine cells. At the crypt-villus junction, the proliferative phase ends, and cells acquire a differentiated phenotype characterized by the synthesis of functionally relevant proteins. The cells continue to migrate up the villi, enter an apoptotic cycle, and are shed into the intestinal lumen ~3 days after their initial appearance on villi. The molecular mechanisms underlying the dynamic processes of intestine-specific gene expression and cellular differentiation during development are poorly understood.

Absorptive enterocytes compose ~95% of epithelial cells on villi and are the cells responsible for the terminal digestion and absorption of nutrients. Lactase-phlorizin hydrolase (LPH), the enzyme critical for the digestion of milk lactose, is an absorptive enterocyte-specific protein that serves as a marker for intestine-specific gene expression and intestinal differentiation (4, 5). In rats, LPH mRNA is detected as early as E18 in the proximal intestine when primitive villi are formed (6). LPH expression is highest at birth and continues to be highly expressed throughout the suckling period. After weaning, LPH expression per enterocyte is reduced and is also restricted to the jejunal and proximal ileum (4, 5). This developmental decline also occurs in humans at around age 5, although a subset of the human population continues to synthesize high levels of LPH throughout adulthood (7, 8). The close correlation between the lactase activity and its mRNA in rats (4, 9) and humans (10, 11) and transcription rate experiments (4) indicate that LPH is regulated mainly by gene transcription.

Transgenic studies indicate that information for enterocyte-specific LPH gene expression in vivo is contained in the 5′-

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1 The abbreviations used are: E, embryonic day; LPH, lactase-phlorizin hydrolase; HNF-1α, hepatocyte nuclear factor-1α; CMV, cytomegalovirus; GST, glutathione S-transferase; EMSA, electrophoretic mobility shift assay; CBP, cAMP-responsive element-binding protein-binding protein.
flanking region (12–14). Identification of specific binding sites within the first 100 bp of 5′-flanking region in the LPH genes of several species has led to the demonstration that GATA, hepatocyte nuclear factor-1α (HNF-1α), and Cdx-2 transcription factors are activators of the LPH promoter (15–21). Due to the close proximity of GATA and HNF-1 binding sites on the LPH promoter and the nearly exclusive coexpression of these two families of transcription factors in the intestinal epithelium (22–29), we hypothesized that members of the GATA and HNF-1 families of transcription factors interact to modulate LPH gene expression.

The GATA family of transcription factors has been implicated in cell lineage differentiation during vertebrate development. Defined by two evolutionarily conserved zinc fingers of the motif Cys-X2-Cys-X7-Cys-X2-Cys that mediate binding to the consensus DNA sequence WGATAR (where W = A or T, and R = A or G), the GATA family is generally categorized into two classes based on expression patterns and amino acid homologies. GATA-1, -2, and -3 are expressed in developing bone marrow cells and are critical for hematopoiesis (30), whereas GATA-4, -5, and -6 have a more diverse pattern of expression that includes the small intestine, heart, liver, lungs, and gonads (22–24, 31–33). The GATA-4, -5, and -6 subfamilies of these two transcription factor families interact to modulate LPH gene expression.

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HNF-1α is a member of a transcription factor family that contains a modified homeodomain and that binds as a dimer to the consensus sequence GTTAA/TATTAAAC (35, 36). Originally thought to be liver-specific, HNF-1α is expressed in the intestinal epithelium (26–29, 37) and has been shown to modulate the promoter of many genes expressed in the intestine (18, 20, 21, 38–45). We have recently shown that GATA subfamily members and HNF-1α synergistically activate the LPH and sucrase-isomaltase promoters (21), suggesting that members of these two transcription factor families interact to produce high levels of enterocyte-specific gene expression.

The goal of these experiments was to characterize the role that GATA and HNF-1α transcription factors play in regulating intestine-specific gene expression by defining the mechanism by which these two transcription factors function to synergistically activate the human LPH promoter. In these studies, the importance of critical structures in GATA factors and HNF-1α, including domains responsible for protein/protein interaction, DNA-binding domains, and activation domains, for cooperative activation was determined. Using GATA-5 as a model, the results of this study reveal that physical association between GATA-5 and HNF-1α is required for the synergistic activation of the human LPH promoter and that this interaction occurs through the C-terminal zinc finger and basic regions of GATA-5 and the homeodomain of HNF-1α. Identification of parallel mechanisms of protein/protein interaction between zinc finger and homeodomain proteins in other vertebrate tissue (46–48) as well as in Drosophila (49, 50) suggests that zinc finger/homeodomain interactions are an efficient mechanism for synergistic activation of gene transcription that has been conserved throughout evolution.

MATERIALS AND METHODS

Plasmids—Previously characterized expression vectors for mouse GATA-4 (G4-CMV) (51) and GATA-5 (G5-CMV) (24) (gifts of M. Parmacek, University of Pennsylvania) and HNF-1α (27) (gift of G. Crabtree, Stanford University) were obtained for these studies. Because the original HNF-1α expression vector replicates inefficiently during bacterial amplification, the HNF-1α coding region was PCR-amplified (5’-ATACCGATCCATGTTTTCTAAGCTGAGCCAGCTG-3’ and 5’-GTATGAATTCCTTACTGGGAAGAGGAGGCCATCTG-3’) and subcloned into the BamHI and EcoRI sites of pcDNA1 (called H1α-CMV). This plasmid was amplified efficiently in Escherichia coli DH5α cells.

For in vivo protein/protein interaction studies, an expression vector for FLAG-tagged GATA-5 (G5-FLAG) was constructed by fusing the GATA-5 coding region to that of FLAG in the pFLAG-CMV-2 expression vector (Sigma). This was done using site-directed mutagenesis (52) by inserting a second EcoRI site at the 5’-side of the GATA-5 coding region (mutagenic oligonucleotide, 5’-CTTTGGTACATGGAATTCGAGAGCT-3’). The GATA-5 coding region was then subcloned in-frame into the EcoRI

| Name | Oligonucleotide | Mutation | Effect |
|------|----------------|----------|--------|
| **GATA mutations** | | | |
| mut 1 | 5′-GGTTTGTGCTTGGAATTCTTGGACCTTGTGAT-3′ | **Stop**, EcoRI | Deletion of C-terminal domain |
| mut 2 | 5′-TGCTGGATACCTCTGGAATTCTTGACCTGAGCTG-3′ | **Stop**, EcoRI | Deletion of C-terminal domain, basic region |
| mut 3 | 5′-GAGGGCTCCGGACATGGATCCGGGCTGGCTGGTC-3′ | BamHI, Start | Deletion of activation domains, Zn finger I |
| mut 4 | 5′-TTGCCCCCCTTCAATGGGATCCATGAGGAGCCAGG-3′ | BamHI, Start | Deletion of activation domains, Zn finger II |
| mut 5 | 5′-GCCACAGGGTCTTAGAACCAGTGTTGC-3′ | C270S | Cysteine to serine substitution in Zn finger II |

**HNF-1α mutations**

| Name | Oligonucleotide | Mutation | Effect |
|------|----------------|----------|--------|
| mut 1 | 5′-GGTGGAGTTCAATTCCAGTTATGATGGAATTC-3′ | **Stop**, EcoRI | Deletion of activation domains |
| mut 2 | 5′-GGTGGAGTTCAATTCCAGTTATGGAATTC-3′ | **Stop**, EcoRI | Deletion of activation domains and partial deletion of the homeodomain |

**FIG. 1. Oligonucleotides used for the introduction of mutations in GATA-5 and HNF-1α.** The name, oligonucleotide sequence, type of mutation, and effect of the mutation are indicated. Uracil templates for both G5-pGEM and H1α-CMV required the use of reverse strand oligonucleotides.
site of pFLAG-CMV-2, oriented by restriction digests, and confirmed by sequencing.

For _in vitro_ protein/protein interaction studies, glutathione 5-transferase (GST) fusion vectors were constructed for GATA-5 (GST-G5) and HNF-1α (GST-H1α). GST-G5 and GST-H1α were made by site-directed mutagenesis using PCR, amplification, and subcloning, respectively, by introducing BamHI and EcoRI sites adjacent to the start and stop codons: GATA-5, 5′-CAACGCTTTGGTACATGATCCCTCCCCGGCGGA-3′ and 5′-GTTGAGCATGTTCCAATTGCTTGCAAGGGA-3′; and HNF-1α, 5′-ATACGGGATACTGTTTCTCAGTGAGCCAGCTG-3′ and 5′-GATGTAATTCCTTACCTGGAAAGGAGGCCATCGT-3′. Both constructs were subsequently subcloned in-frame into the BamHI and EcoRI sites of pGEX-2TK (Promega) and confirmed by sequencing.

For transfection studies, the human LPH promoter containing 118 bp of 5′-flanking region was fused to the human growth hormone reporter (called h118wt) as previously described (21). This region contains two GATA sites and an HNF-1 site as previously described (21). Transfection 

**Fig. 2. GATA-5 and HNF-1α physically associate in _in vitro_.** EMSAs using the rat β-fibronectin HNF-1α site as a probe (53) were carried out on nuclear extracts from COS-7 cells that were cotransfected with G5-FLAG and H1α-CMV and immunoprecipitated with beads coated with anti-FLAG antibodies. A major protein-DNA complex (arrowhead) was detected (lane 2) that formed a supershift complex (SC) using an anti-HNF-1α antibody (lane 3). Nuclear extracts from COS-7 cells cotransfected with FLAG-CMV and H1α-CMV were used as a negative control (lane 4).
active metallothionein I promoter fused to the human growth hormone gene.

Statistics—The t test or one-way analysis of variance was employed in all statistical analyses using InStat software (GraphPAD Software, Inc.). Multiple comparisons were carried out by the Dunnett multiple comparison test.

RESULTS

GATA-5 and HNF-1α synergistically activate the human LPH promoter (21), suggesting that these two transcription factors physically associate. To test the hypothesis that GATA-5 and HNF-1α physically associate in vivo, COS-7 cells were cotransfected with G5-FLAG and H1α-CMV, and FLAG-associated proteins were immunoprecipitated from nuclear extracts using beads coated with anti-FLAG antibodies. Detection of HNF-1α in the G5-FLAG immunoprecipitate indicates GATA-5/HNF-1α interactions. As shown by EMSAs using an HNF-1-binding site as a probe (Fig. 2), a protein-DNA complex was identified that supershifted with an anti-HNF-1α antibody, demonstrating that HNF-1α was present in the anti-FLAG immunoprecipitate. HNF-1α was not detected in nuclear extracts from COS-7 cells cotransfected with FLAG-CMV and H1α-CMV, indicating that HNF-1α does not bind to FLAG alone. These data demonstrate that HNF-1α physically associates with GATA-5 in vivo.

To characterize critical domains in GATA-5 necessary for physical association and functional cooperativity with HNF-1α, mutations were introduced into GATA-5 to delete or disrupt specific structures (Fig. 3A). The capacity of wild-type and mutant GATA-5 proteins to associate physically with HNF-1α in vitro was then tested by GST pull-down assays using GST fused to HNF-1α. HNF-1α was not pulled down by GST alone (lane 7). All proteins used in the GST pull-down assays were synthesized as shown by direct loading of TNT products (lower panel). C, the C-terminal zinc finger and basic regions are critical for binding to DNA. EMSAs were carried out using the Xenopus intestinal fatty acid-binding protein GATA site as a probe (25) and wild-type and mutant GATA-5 proteins as indicated (lanes 2–9). Wild-type GATA-5 formed a complex (lane 2) that was competed with a specific oligonucleotide (S; lane 3), but not with a nonspecific oligonucleotide (N; lane 4). Wild-type GATA-5 and GATA-5 mut1, mut3, and mut4 all bound to DNA, whereas GATA-5 mut2 and mut5 did not.

Fig. 3. C-terminal zinc finger and basic regions of GATA-5 are necessary for physical association with HNF-1α and binding to DNA. A, schematic representation of wild-type and mutant GATA-5 proteins. GATA-5 contains two N-terminal activation domains (AD I and AD II), two zinc fingers (ZnI, N-terminal zinc finger; and ZnII, C-terminal zinc finger), and a basic region (B) near the C terminus (24). The C-terminal domain is deleted in mut1, whereas the C-terminal domain and basic region are deleted in mut2. Both activation domains are deleted in mut3, whereas both activation domains and the N-terminal zinc finger are deleted in mut4. GATA-5 mut5 is a cysteine-to-serine substitution in the C-terminal zinc finger at amino acid 270. B, the C-terminal zinc finger and basic regions of GATA-5 are necessary for physical association with HNF-1α. GST pull-down assays (upper panel) were carried out using GST-H1α incubated with labeled, in vitro transcribed and translated wild-type (WT) (lane 1) and mutant (lanes 2–6) GATA-5. Wild-type GATA-5 and GATA-5 mut1, mut3, and mut4 were pulled down by GST-H1α, whereas GATA-5 mut2 and mut5 were not. GATA-5 was not pulled down by GST alone (lane 7). All proteins used in the GST pull-down assays were synthesized as shown by direct loading of TNT products (lower panel). C, the C-terminal zinc finger and basic regions are critical for binding to DNA. EMSAs were carried out using the Xenopus intestinal fatty acid-binding protein GATA site as a probe (25) and wild-type and mutant GATA-5 proteins as indicated (lanes 2–9). Wild-type GATA-5 formed a complex (lane 2) that was competed with a specific oligonucleotide (S; lane 3), but not with a nonspecific oligonucleotide (N; lane 4). Wild-type GATA-5 and GATA-5 mut1, mut3, and mut4 all bound to DNA, whereas GATA-5 mut2 and mut5 did not.
mut4) were pulled down by GST-H1α. In contrast, GATA-5 mut2, in which the basic region and C-terminal domain are deleted, and GATA-5 mut5, in which the structure of the C-terminal zinc finger is disrupted, were not pulled down by GST-H1α. GST alone did not pull down wild-type GATA-5, indicating that GST does not interact with GATA-5. Direct loading of labeled TnT products (Fig. 3B, lower panel) indicates that proteins of predicted sizes were synthesized. These data demonstrate that the C-terminal zinc finger and basic regions of GATA-5 are required for physical association with HNF-1α.

To map DNA-binding domains in GATA-5, EMSAs were carried out using a GATA-binding site as a probe and in vitro synthesized wild-type and mutant GATA-5 proteins (Fig. 3C). A protein-DNA complex was formed with wild-type GATA-5 that was competed away by a specific oligonucleotide, but not by a nonspecific oligonucleotide, indicating that GATA-5 binds the DNA specifically. GATA-5 mut1, mut3, and mut4 bound DNA, but GATA-5 mut2 and mut5 did not. These data are parallel to the GST pull-down experiments demonstrating that the domains critical for GATA-5/DNA interaction also map to the C-terminal zinc finger and basic regions. Thus, regions in GATA-5 that mediate protein/protein interaction with HNF-1α and DNA binding are co-localized to the C-terminal zinc finger and basic regions.

The functional importance of specific structures in GATA-5 for synergistic GATA-5/HNF-1α activation of the human LPH promoter was tested by transient cotransfection assays using wild-type or mutant GATA-5 and wild-type HNF-1α expression vectors (Fig. 4). To test specific effects of transfected wild-type and mutant GATA-5 and HNF-1α, a model system utilizing cells that do not synthesize appreciable amounts of these factors was necessary. HeLa cells provided such a model system, as previously described in Caco-2 cells (21), whereas GATA-5 alone only minimally activated this promoter. The human LPH promoter was synergistically activated by a combination of GATA-5 plus HNF-1α, as indicated by a transcriptional activity that was ~3-fold greater than the sum of the individual transcriptional activities of GATA-5 and HNF-1α alone (indicated by the dashed line). GATA-5 mut1, mut3, and mut4 all demonstrated synergistic activation of the human LPH promoter when cotransfected with HNF-1α as indicated by a mean activation that extends to the right of the dashed line. Noteworthy, however, is that synergy, although present, was greatly reduced with mut3 and mut4, which do not contain GATA-5 activation domains. GATA-5 mut2 and mut5, which failed to show synergistic activation of the human LPH promoter, were used throughout the remainder of this study. pRC-CMV was used as a negative control. The human LPH promoter (h118wt) was independently activated by HNF-1α, as previously described in Caco-2 cells (21), whereas GATA-5 alone only minimally activated this promoter. The human LPH promoter was synergistically activated by a combination of GATA-5 plus HNF-1α, as indicated by a transcriptional activity that was ~3-fold greater than the sum of the individual transcriptional activities of GATA-5 and HNF-1α alone (indicated by the dashed line). GATA-5 mut1, mut3, and mut4 all demonstrated synergistic activation of the human LPH promoter when cotransfected with HNF-1α as indicated by a mean activation that extends to the right of the dashed line. Noteworthy, however, is that synergy, although present, was greatly reduced with mut3 and mut4, which do not contain GATA-5 activation domains. GATA-5 mut2 and mut5, which failed to show synergistic activation of the human LPH promoter, were used throughout the remainder of this study. pRC-CMV was used as a negative control. The human LPH promoter (h118wt) was independently activated by HNF-1α, as previously described in Caco-2 cells (21), whereas GATA-5 alone only minimally activated this promoter. The human LPH promoter was synergistically activated by a combination of GATA-5 plus HNF-1α, as indicated by a transcriptional activity that was ~3-fold greater than the sum of the individual transcriptional activities of GATA-5 and HNF-1α alone (indicated by the dashed line). GATA-5 mut1, mut3, and mut4 all demonstrated synergistic activation of the human LPH promoter when cotransfected with HNF-1α as indicated by a mean activation that extends to the right of the dashed line. Noteworthy, however, is that synergy, although present, was greatly reduced with mut3 and mut4, which do not contain GATA-5 activation domains. GATA-5 mut2 and mut5, which failed to show synergistic activation of the human LPH promoter, were used throughout the remainder of this study. pRC-CMV was used as a negative control. The human LPH promoter (h118wt) was independently activated by HNF-1α, as previously described in Caco-2 cells (21), whereas GATA-5 alone only minimally activated this promoter. The human LPH promoter was synergistically activated by a combination of GATA-5 plus HNF-1α, as indicated by a transcriptional activity that was ~3-fold greater than the sum of the individual transcriptional activities of GATA-5 and HNF-1α alone (indicated by the dashed line). GATA-5 mut1, mut3, and mut4 all demonstrated synergistic activation of the human LPH promoter when cotransfected with HNF-1α.
and mutant HNF-1 transient cotransfection assays using h118wt and wild-type (wt) and mutant expression vectors for GATA-5 and HNF-1α. pRC-CMV was used as a negative control. Wild-type and mutant GATA-5 and HNF-1α proteins are as indicated in Figs. 3A and 5A, respectively. The dashed lines indicate the sum of the transcriptional activities of wild-type and mutant GATA-5 (G5) and HNF-1α (H1α) expression vectors individually cotransfected with h118wt for that specific combination of expression vectors. Transcriptional activities that extend to the right of the dashed lines indicate synergistic activation of the h118wt promoter. Data are means ± S.E. (n = 4). *, p < 0.05 compared with h118wt cotransfected with wild-type GATA-5 and HNF-1α. AD, activation domain.

Fig. 6. Activation domains of HNF-1α are required for synergistic activation of the human LPH promoter. Transient cotransfection assays were carried out in HeLa cells using h118wt and wild-type (wt) and mutant expression vectors for GATA-5 and HNF-1α. pRC-CMV was used as a negative control. Wild-type and mutant GATA-5 and HNF-1α proteins are as indicated in Figs. 3A and 5A, respectively. The dashed lines indicate the sum of the transcriptional activities of wild-type and mutant GATA-5 (G5) and HNF-1α (H1α) expression vectors individually cotransfected with h118wt for that specific combination of expression vectors. Transcriptional activities that extend to the right of the dashed lines indicate synergistic activation of the h118wt promoter. Data are means ± S.E. (n = 4). *, p < 0.05 compared with h118wt cotransfected with wild-type GATA-5 and HNF-1α. AD, activation domain.

DNA-binding domains in HNF-1α were mapped by EMSAs using wild-type and mutant HNF-1α synthesized in vitro (Fig. 5C). Wild-type HNF-1α formed a protein-DNA complex that supershifted using an anti-HNF-1α antibody, indicating that the complex contains HNF-1α. HNF-1α mut1 bound DNA (Fig. 5C, lane 4), but HNF-1α mut2 did not (lane 5). These data demonstrate that an intact homeodomain in HNF-1α is critical for HNF-1α/DNA binding. Thus, regions in HNF-1α that mediate protein/protein interaction with GATA-5 and DNA binding are co-localized to the homeodomain.

The role of HNF-1α activation domains in the cooperative activation of the human LPH promoter was characterized by transient cotransfection assays using h118wt and wild-type and mutant HNF-1α (mut1). For comparison, wild-type GATA-5 and GATA-5 mut3, which contains a deletion of the GATA-5 activation domains, were also used. This allowed comparative analyses of the activation domains of GATA-5 and HNF-1α together (Fig. 6). Consistent with previous data (Fig. 4), deletion of GATA-5 activation domains reduced, but did not eliminate, functional synergy. In contrast, deletion of the HNF-1α activation domains eliminated independent as well as cooperative activation of the human LPH promoter. Lack of activation by HNF-1α mut1 was not due to the inability to associate with GATA-5 or to inefficient DNA binding because these functions remained intact for this mutant (Fig. 5).

Because domains in GATA-5 and HNF-1α that confer physical association are co-localized with DNA-binding domains, it is not possible by mutagenesis of GATA-5 or HNF-1α to differentiate between the importance of GATA/HNF-1α interaction and DNA binding. However, it is possible to independently determine the importance of protein-DNA interaction by introducing mutations that disrupt DNA interaction into the binding sites in the human LPH promoter. As shown in Fig. 7, transient cotransfection assays were carried out using h118wt and mutant promoter-reporter constructs that have the GATA- and HNF-1-binding sites either intact or mutated. As shown by the middle bar, the wild-type human LPH promoter cotransfected with GATA-5 and HNF-1α demonstrated synergistic activation. GATA-5 and HNF-1α cotransfected with h118mG1G2 demonstrated synergistic activation, although the transcriptional activity was reduced from that of the wild-type promoter. However, constructs containing mutations in the HNF-1-binding site alone (h118mH) or together with the mutated GATA-binding sites (h118mG1G2H) did not show synergistic activation, and their transcriptional activities were significantly lower than that of the wild-type promoter (p < 0.05). These data demonstrate that HNF-1-binding sites in the promoter are necessary for GATA-5/HNF-1α cooperative activation.

Sequence alignment of GATA-4 and GATA-5 reveals 85% homology in the C-terminal zinc finger and basic regions (24), which are the domains responsible for interaction with HNF-1α. To test the hypothesis that GATA-4 and HNF-1α are also capable of physically associating and cooperatively activating the human LPH promoter, GST pull-down and transient cotransfection assays were carried out. As indicated by the presence of a labeled protein on SDS-polyacrylamide gels (Fig. 8A, upper panel), GATA-4 was pulled down with GST-H1α, similar to GATA-5. Immunoprecipitations were carried out using antibodies for GATA-4 and GATA-5, demonstrating that the TnT reactions resulted in the synthesis of authentic GATA-4 and GATA-5 proteins (Fig. 8A, lower panel). These data suggest that GATA-4, like GATA-5, physically associates with HNF-1α.
Transient cotransfection assays carried out in HeLa cells (Fig. 8B) demonstrated that GATA-4 and HNF-1α independently and synergistically activated the human LPH promoter. These data suggest that GATA-4 is capable of interacting with HNF-1α by a mechanism similar to that described for GATA-5.

DISCUSSION

The GATA zinc finger and HNF-1 homeodomain families have been implicated as regulators of tissue-specific gene expression (34, 37). The mRNAs of members of both of these transcription factor families are detected in the foregut as early as E9.5 for GATA factors and E10.5 for HNF-1α. Both are expressed in the intestinal epithelium throughout adulthood, suggesting a critical role for these two families of transcription factors in intestinal function (29, 34, 56). This report shows for the first time that physical association between members of each of these transcription factor families, namely GATA-4 or GATA-5 and HNF-1α, results in the cooperative activation of the promoter of an intestine-specific gene, LPH, suggesting functional convergence of two critical intestinal transcriptional regulatory pathways to maintain high levels of intestine-specific gene expression.

We have previously shown that GATA-5 and HNF-1α synergistically activate the human LPH promoter in the Caco-2 intestinal cell line (21). In the present report, we have demonstrated that this synergistic activation requires physical association between GATA-5 and HNF-1α and that this interaction is mediated by the C-terminal zinc finger and basic regions of GATA-5 and the homeodomain of HNF-1α. Our data further demonstrate that HNF-1α activation domains are necessary not only for independent HNF-1α activation, but also for synergistic activation with GATA-5. Deletion of GATA-5 activation domains results, however, in a reduction, but not an elimination, of synergy, suggesting that the GATA-5 activation domains are not necessary, but serve as additional activators for maximal synergy. Mutational analysis of the human LPH promoter further revealed that intact HNF-1-binding sites, but not GATA-binding sites, are necessary for synergistic activation of the human LPH promoter (Fig. 7). As shown in Figs. 3 and 4, GATA-5 mut5 (C270S) failed to activate the human LPH promoter by GATA-5 mut5 must be due to its inability to bind DNA, and synergistically activate h118wt. However, promoter mutation experiments (Fig. 7) revealed that GATA binding to DNA was not necessary for functional synergy. Thus, failure to synergistically activate the LPH promoter by GATA-5 mut5 must be due to its inability to physically associate with HNF-1α, rather than its inability to bind DNA. Taken together, these data demonstrate that physical interaction is required for functional synergy. Based on these data, we propose the following model: GATA/HNF-1α synergy is mediated by HNF-1α through its activation domains, which are oriented for high levels of activation through a combination of binding to DNA and physical association with GATA factors (Fig. 9). GATA/HNF-1α interactions might unmask the HNF-1α activation domains either by a conformational change or by recruitment of additional proteins that modulate transcriptional activity.

Interaction between zinc fingers and homeodomains may be
a critical biological mechanism for gene regulation. For example, similar to the model presented here is the well characterized model for the synergistic activation of cardiac promoters by GATA-4 and Nkx2.5 (47, 48, 57–60), a homeodomain-containing transcription factor like HNF-1α. In this model, the C-terminal zinc finger and basic regions of GATA-4 and the homeodomain of Nkx2.5 are required for physical association, which, in turn, is necessary for synergistic activation of several genes (47, 48, 57). Furthermore, intact Nkx2.5-binding sites on cardiac promoters are required for synergistic activation (47, 48, 57). HNF-1α cooperatively activates the sodium-glucose transporter (SGLT1) with SP-1, a zinc-finger-containing protein (44). In Drosophila, interactions between the zinc finger of Ftz-F1, a member of the nuclear receptor superfamily, and the homeodomain of the fushi tarazu Ftz protein result in the cooperative activation of the engrailed gene (49, 50). These findings are parallel to the GATA-5/HNF-1α model proposed here and suggest an evolutionarily conserved structure/function relationship that preserves a mechanism of cooperative activation of multiple genes in diverse tissues.

HNF-1α has been shown to interact through its homeodomain with other transcription factors in the intestine. A report by Sakaguchi et al. (45) suggested that HNF-1α is able to enhance claudin-2 promoter activity only in the presence of another member of the homeodomain transcription factor family, Cdx-2. In addition, Mitchelmore et al. (20) demonstrated that HNF-1α physically interacts with Cdx-2 to cooperatively activate the pig LPH promoter. Interestingly, similar to the GATA-5/HNF-1α model presented in this study, HNF-1α, but not Cdx-2, must bind to the DNA for cooperative activation. Because HNF-1α binds to the DNA as a dimer (35, 36), it remains possible that for the specific regulation of the LPH gene in vivo, all three factors form a trimolecular complex whereby the HNF-1α dimer binds GATA factors, Cdx-2, or a combination of both. This hypothesis is consistent with our previous studies (21), which show that the human LPH and sucrase-isomaltase promoters demonstrate synergistic activation when cotransfected with GATA-5, HNF-1α, and Cdx-2 together. A similar mechanism has also been reported for the cardiac α-actin promoter, which is up-regulated through combinatorial interactions of at least three cardiac tissue-enriched transcription factors, GATA-4, Nkx2.5 (which also forms dimers, like HNF-1α), and serum response factor (48). HNF-1α may also recruit coactivators such as CBP and p300/CBP-associated factor, forming a trimolecular complex that activates transcription by coupling nucleosome modification with recruitment of proteins for the general transcription machinery (61). Together, these data suggest that HNF-1α has the ability to physically associate with diverse proteins and to act as a linker to the DNA to synergistically activate intestinal gene promoters. The magnitude of cooperative activation may be altered by the abundance of different transcription factors and their affinity for HNF-1α.

Physical association between GATA family members and HNF-1α is necessary for the synergistic activation of the human LPH promoter, providing a mechanism by which tissue-restricted transcription factors can interact to attain high levels of tissue-specific gene expression. However, although GATA (22, 24) and HNF-1α (29) transcription factors are co-localized in the intestinal epithelial, where LPH expression is high (5, 6), they are also coexpressed in primitive intestinal epithelial cells prior to the onset of LPH gene expression (23, 24, 32, 37). We therefore hypothesize that coexpression of GATA factors and HNF-1α is necessary, but not sufficient, for high levels of cell-specific LPH gene expression. Interaction with other tissue-restricted transcription factors and cofactors such as SP-1 (44), FOG-2 (62, 63), Cdx-2 (20), and the dimerization cofactor of HNF-1α (64) as well as other information further upstream in the LPH promoter (18, 65) must be considered when characterizing specific expression of the LPH gene in vivo.

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