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GATA4 and GATA6 regulate intestinal epithelial cytodifferentiation during development

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Abstract

The intestinal epithelium performs vital roles in organ function by absorbing nutrients and providing a protective barrier. The zinc-finger containing transcription factors GATA4 and GATA6 regulate enterocyte gene expression and control regional epithelial cell identity in the adult intestinal epithelium. Although GATA4 and GATA6 are expressed in the developing intestine, loss of either factor alone during the period of epithelial morphogenesis and cytodifferentiation fails to disrupt these processes. Therefore, we tested the hypothesis that GATA4 and GATA6 function redundantly to control these aspects of intestinal development. We used Villin-Cre, which deletes specifically in the intestinal epithelium during the developmental period of villus development and epithelial cytodifferentiation, to generate *Gata4Gata6* double conditional knockout embryos. Mice lacking GATA4 and GATA6 in the intestinal epithelium died within 24 hours of birth. At E18.5, intestinal villus architecture and epithelial cell populations were altered. Enterocytes were lost, and goblet cells were increased. Proliferation was also increased in GATA4-GATA6 deficient intestinal epithelium. Although villus morphology appeared normal at E16.5, the first time at which both *Gata4* and *Gata6* were efficiently reduced, changes in expression of markers of enterocytes, goblet cells, and proliferative cells were detected. Moreover, goblet cell number was increased at E16.5. Expression of the Notch ligand *Dll1* and the Notch target *Olfm4* were reduced in mutant tissue indicating decreased Notch signaling. Finally, we demonstrated that GATA4 occupies chromatin near the *Dll1* transcription start site suggesting direct regulation of *Dll1* by GATA4. We demonstrate that GATA4 and GATA6 play an essential role in maintaining proper intestinal epithelial structure and in regulating intestinal epithelial cytodifferentiation. Our data highlight a novel role for GATA factors in fine tuning Notch signaling during intestinal epithelial development to repress goblet cell differentiation.

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Transcript profiling: Microarray data from this study have been deposited into NCBI Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo) and are accessible through GEO series accession number GSE49471.

Introduction

The intestinal epithelium plays a central role in orchestrating organ function through nutrient absorption and by providing a barrier between the environment and underlying tissues. During embryonic development, epithelial morphogenesis and cytodifferentiation in midgut endoderm produce a precisely structured epithelium composed of specialized cell types that perform these functions (Spence et al., 2011). In mouse, between embryonic day 14 (E14) and birth, the immature pseudostratified epithelium of the gut converts to a simple columnar epithelium covering mucosal projections known as villi (Grosse et al., 2011). Coincident with epithelial morphogenesis, progenitor cells differentiate into absorptive or secretory cell types. As the epithelium remodels, proliferative progenitor cells become restricted to intervillus regions, which mark the future sites of crypts where intestinal stem cells and secretory Paneth cells will reside (Spence et al., 2011).

One family of factors implicated in enterocyte development is the GATA family of zinc-finger DNA binding transcription factors, specifically GATA4 and GATA6. Both GATA4 and GATA6 are expressed in midgut endoderm during development and continue to be expressed in the small intestinal epithelium throughout adulthood although in differing patterns (Koutsourakis et al., 1999; Bosse et al., 2006; Bosse et al., 2007; Watt et al., 2007; Battle et al., 2008; Beuling et al., 2011). Epithelial cells of duodenum and jejunum express GATA4, whereas those of the ileum lack GATA4 (Bosse et al., 2006; Battle et al., 2008). GATA6, however, is expressed in all regions of the small intestinal epithelium (Fang et al., 2006). Because *Gata4*−/− and *Gata6*−/− mice die during embryogenesis prior to organ development (Kuo et al., 1997; Molkentin et al., 1997; Morrisey et al., 1998; Koutsourakis et al., 1999), it was necessary to use a conditional knockout approach to study their function in the small intestine. Using Villin-Cre, which directs robust intestinal epithelium-specific recombination during development beginning at the time of villus morphogenesis and epithelial cytodifferentiation (Madison et al., 2002), we previously eliminated GATA4 from the intestine. We found that although GATA4 is dispensable for proper intestinal development when deleted during the period of epithelial morphogenesis and cytodifferentiation with Villin-Cre, it is essential for jejunal function (Battle et al., 2008). Mice lacking GATA4 in the jejunal epithelium displayed severe defects in fat and cholesterol absorption. Moreover, mutant jejunum lost expression of many jejunal-specific genes and gained expression of many ileal-specific genes suggesting that GATA4 plays a key role in determining jejunal enterocyte identity. Conditional knockout of GATA4 in adult mouse small intestine using tamoxifen-inducible Villin-Cre resulted in a similar phenotype (Bosse et al., 2006). Loss of GATA6 from the intestinal epithelium using Villin-Cre also fails to disrupt embryonic intestinal development (Battle lab, unpublished data). Conditional knockout of *Gata6* in adult mouse small intestinal epithelium using tamoxifen-inducible Villin-Cre alters ileal epithelial cell populations including a reduction of proliferative, enteroendocrine, and Paneth cells and an increase in goblet cells (Beuling et al., 2011). Loss of *Gata6* in the ileum also causes changes in the ileal enterocyte-specific gene expression pattern, shifting it toward a more distal colon-like pattern (Beuling et al., 2011).

The finding that GATA4 and GATA6 are expressed in the developing intestine, yet loss of either factor alone during the period of epithelial morphogenesis and cytodifferentiation fails

to disrupt intestinal development, suggests that these factors function redundantly during this period of small intestinal development. Redundancy in GATA4-GATA6 function has been demonstrated during development of other organs. For example, loss of either GATA4 or GATA6 in the heart results in subtle phenotypes whereas loss of both factors causes acardia (Zhao et al., 2008). Studies examining GATA4 and GATA6 in pancreatic development confirm a similar model of GATA function. Loss of either GATA4 or GATA6 causes minor defects, whereas elimination of both results in pancreatic agenesis (Carrasco et al., 2012; Xuan et al., 2012). Further supporting redundant function of GATA factors in the small intestinal epithelium, conditional knockout of *Gata4* and *Gata6* in adult mouse small intestinal epithelium using tamoxifen-inducible Villin-Cre causes changes in the duodenum and jejunum similar to those seen in GATA6-deficient ileum including a reduction of proliferative, enteroendocrine, and Paneth cells and an increase in goblet cells (Beuling et al., 2011). The impact of loss of both GATA4 and GATA6 on intestinal development, however, is unknown. Therefore, to test the hypothesis that GATA4 and GATA6 regulate a common set of genes to control intestinal development, *Gata4Gata6* double conditional knockout (*G4G6* dcKO) mice were generated using Villin-Cre. We found that unlike single *Gata4* or *Gata6* Villin-Cre cKO mice or mice with deletion of both *Gata4* and *Gata6* in adult intestine, elimination of both *Gata4* and *Gata6* during development resulted in death within 24 hours of birth. Both epithelial architecture and cell type allocation were affected in GATA4-GATA6 deficient intestine. Although villi emerged normally in mutants, villus structure was abnormal at E18.5 with mutant tissue containing scarce short, broad villi. We observed a decrease in enterocytes and an increase in proliferating cells and goblet cells. Reduced expression of the Notch ligand *Dll1* (Pellegrinet et al., 2011) and the Notch downstream target *Olfm4* (VanDussen et al., 2012) suggested defective Notch signaling, and we found that GATA4 occupied binding sites within the *Dll1* gene in the intestinal epithelium. We conclude that GATA4 and GATA6 play an essential role in regulating intestinal epithelial structure and cytodifferentiation. Moreover, the data we present suggest a role for GATA factors in intestinal epithelial cell fate decisions by modulating Notch signaling through regulation of *Dll1*.

Materials and Methods

Animals

Gata4^{loxP}(*Gata4*$^{tm1.1Sad}$), *Gata6*^{loxP}(*Gata6*$^{tm2.1Sad}$), *Gata6*$^{-}$(*Gata6*$^{tm2.2Sad}$), *Villin-Cre*(Tg(*Vil-cre*)997Gum), *Gata4*^{flbio}(*Gata4*$^{tm3.1Wtp}$) and *Rosa26*^{BirA}(*Gt(ROSA)26Sor*$^{tm1(birA)Mejr}$) mice were used (Madison et al., 2002; Watt et al., 2004; Driegen et al., 2005; Sodhi et al., 2006; He et al., 2012). Embryonic mice were generated by timed mating considering noon on the day of a vaginal plug as E0.5. Genotypes were determined by PCR of tail tip or ear punch DNA following a standard protocol. Primers are listed in Supplemental Table 1. For proliferation studies, 200 μg 5-ethynyl-2′deoxyuridine (EdU) was administered by intraperitoneal injection 3h prior to euthanizing animals. The Medical College of Wisconsin's Animal Care Committee approved all animal procedures.

Intestinal Epithelial Cell Isolation

Whole small intestine harvested from control and *G4G6* dcKO E16.5, E17.5, or E18.5 embryos was cut along its longitudinal axis and incubated in cell dissociation buffer (BD Biosciences, San Jose, CA) for 6h at 4°C with gentle agitation to release epithelial cells (Madison et al., 2005; Li et al., 2007). Total RNA prepared from epithelial cells was used for gene array and qRT-PCR analyses.

Reverse Transcription Polymerase Chain Reaction

cDNA was generated from DNase treated RNA harvested from intestinal epithelial cells isolated from the whole small intestine (E16.5–E18.5) or from intact jejunal tissue harvested from the midpoint of small intestine (E15.5) as previously described (Duncan et al., 1997; Bondow et al., 2012). Supplemental Tables 1 and 2 contain primer sequences and TaqMan assay identifiers, respectively. qRT-PCR data were analyzed using DataAssist software (Applied Biosystems, Carlsbad, CA). *Gapdh* was used for normalization. Each gene was assayed in at least three independent experiments using cDNA from three control and three mutant intestines. Error bars represent standard error of the mean (SEM).

Oligonucleotide Array Analysis

Mouse Gene 1.0 ST arrays (Affymetrix, Santa Clara, CA) were used to determine gene expression changes between intestinal epithelial cells harvested from three control and three *G4G6* dcKO embryos at E18.5 as previously described (Bondow et al., 2012). To be considered significantly changed between groups, we required an expression change of ≥2.0-fold, p 0.05 (Supplemental Table 3).

Histochemistry, Immunohistochemistry, and Immunofluorescence

Histochemistry, immunohistochemistry, and immunofluorescence were performed as previously described using jejunal tissue harvested from the midpoint of each embryonic small intestine (Duncan et al., 1997; Bondow et al., 2012). For each stain, four to six sections from three controls and three mutant animals were used. See Supplemental Table 4 for antibody details.

Biotin-mediated chromatin immunoprecipitation (bio-ChIP)

Epithelial cells from the jejunum of 6-week-old $Gata4^{flbio/flbio}::Rosa26^{BirA/BirA}$ (n=4) and $Rosa26^{BirA/BirA}$ (n=4) animals were obtained using a standard method (Driegen et al., 2005; Guo et al., 2009; He et al., 2012). Cells fixed in 1% formaldehyde for 15 min at room temperature followed by quenching in 1M glycine for 5 min were sonicated using a Misonix Sonicator 3000 (Misonix Incorporated, Farmingdale, NY). Bio-ChIP was performed as previously described with the exception that magnetic beads were washed four times with 2% SDS and twice with high salt buffer (He and Pu, 2010). Chromatin harvested from each animal was subjected to at least two independent pull-down experiments. For each pull-down experiment, occupancy of GATA4 at the *Dll1* locus was detected by PCR using primers flanking predicted GATA4 binding sites and α-^{32}P-dATP. As a negative control, PCR was also performed using primers to a region upstream of the *Dll1* promoter lacking predicted GATA binding sites. Primer sequences are listed in Supplemental Table 1. PCR

products separated in 4% polyacrylamide gels were visualized by autoradiography. Input chromatin was diluted 1:10 prior to PCR amplification.

Results

Loss of GATA4 and GATA6 disrupts the developing intestinal epithelium

To test the hypothesis that GATA4 and GATA6 play redundant roles during intestinal development, we eliminated *Gata4* and *Gata6* specifically in the intestinal epithelium during embryonic development using Villin-Cre. Among 127 three-week-old animals genotyped, we failed to identify animals with a *G4G6* dcKO genotype (*Gata4*$^{loxP/loxP}$*Gata6*$^{loxP/-}$ *Villin-Cre*) demonstrating that intestinal expression of GATA4 and GATA6 is required for survival. We observed litters and identified a subset of pups dying within 24 hours of birth. Genotyping revealed these to be *G4G6* dcKO animals. Genotyping of E18.5 embryos demonstrated the presence of Mendelian ratios of *G4G6* dcKO embryos indicating that lethality occured during the early postnatal period.

Because *G4G6* dcKO mice died within 24 hours of birth, we began our study by investigating the phenotype at E18.5. Examination of intestines from E18.5 control (*Gata4*$^{loxP/loxP}$*Gata6*$^{loxP/+}$ or *Gata4*$^{loxP/+}$*Gata6*$^{loxP/+}$) and *G4G6* dcKO embryos showed that mutant small intestines were dilated compared with controls likely because mutant tissue contained more fluid compared with control tissue (Figure 1A). We measured the length of control and GATA4-GATA6 deficient small intestine and found that mutant small intestine was 20% shorter than control small intestine (Figure 1B). To verify GATA4 and GATA6 loss in *G4G6* dcKOs, we analyzed steady-state mRNA and protein levels by RT-PCR and immunohistochemistry. Both GATA4 and GATA6 mRNA and protein were absent in E18.5 mutant small intestine (Figure 1C, D).

Because GATA4 and GATA6 are co-expressed only in the proximal intestine (duodenum and jejunum) and our aim was to determine the extent to which GATA4 and GATA6 function overlaps to control small intestinal development, we performed morphological analyses including histology, immunohistochemistry, and immunofluorescence using tissue from the jejunum of *G4G6* dcKOs. Histological examination of the jejunum showed defective intestinal epithelial architecture in *G4G6* dcKO embryos (Figure 1E). Epithelial cells of villi in control tissue appeared columnar with basal nuclei, whereas epithelial cells on villi in mutant tissue appeared rounded with centrally located nuclei (Figure 1E, inset). Moreover, in contrast to control tissue, which contained numerous well-developed villi, mutant tissue contained few blunted, irregular villi (Figure 1E). We also surveyed villus structure in duodenal tissue of *G4G6* dcKOs and found it to be similarly abnormal in mutants (Supplemental Figure 1). We further compared villus structure in the jejunum between controls and mutants by staining tissue with an antibody for the mesenchymal marker PDGFRA to confirm the presence of lamina propria mesenchyme underlying villus epithelium and found comparable expression and localization of PDGFRA+ mesenchyme between controls and mutants (Figure 1F). Moreover, although villi of mutants were abnormal in shape and number, clusters of PDGFRA+ cells were present at remaining villus tips; such clusters are required for villus morphogenesis (Karlsson et al., 2000; Walton et al., 2012).

Abnormal villus architecture suggested the possibility that epithelial cells were decreased in mutants compared with controls. We stained tissue for the epithelial cell-specific nuclear marker HNF4A and counted HNF4A+ cells per section; we found no difference in the average number of epithelial cells present in mutant tissue compared with control tissue (Figure 1G). HNF4A staining further revealed that mutant epithelial cells within intervillus regions were densely packed containing thin, elongated nuclei reminiscent of the pseudostratified epithelium present during early intestinal development (Figure 1G, inset).

Because previous studies of GATA4 and GATA6 in the intestine demonstrated that overall intestinal cell fate is normally specified and maintained in several different models of intestinal GATA4 and/or GATA6 knockout (Bosse et al., 2006; Battle et al., 2008; Beuling et al., 2011), we did not expect to find that elimination of both GATA4 and GATA6 during development would alter general intestinal epithelial cell fate. Moreover, Villin-Cre is not active in the intestine at the time of intestinal specification. Therefore, to confirm that general intestinal epithelial cell identity was intact in GATA4-GATA6 deficient small intestine, we analyzed the steady-state mRNA and protein levels of CDX2, one of the earliest markers of intestinal fate, by qRT-PCR and immunohistochemistry. We found no decrease in CDX2 in mutants compared with controls (Figure 1H). In fact, although RNA levels were unchanged, CDX2 protein staining was more intense in mutants compared with controls (Figure 1H).

Loss of GATA4 and GATA6 in the small intestinal epithelium alters epithelial cell populations at E18.5

GATA4 and GATA6 function as transcription factors suggesting that the observed defects reflect changes in the intestinal gene expression profile in their absence. To understand mechanisms of GATA function in the intestine, we performed Affymetrix oligonucleotide array analysis to compare gene expression profiles between the intestinal epithelium of control and *G4G6* dcKO E18.5 embryos. We identified 367 genes with expression increased ≥ 2.0 fold ($P \leq 0.05$) and 403 genes with expression decreased ≥ 2.0 fold ($P \leq 0.05$) (Supplemental Table 3). We used Ingenuity Pathway Analysis (IPA) software to categorize genes with altered expression in mutants and found lipid metabolism, small molecule biochemistry, molecular transport, carbohydrate metabolism, and vitamin and mineral metabolism as the top five biological functions affected (Supplemental Figure 2). These data suggested a defect in the enterocyte population in GATA4-GATA6 deficient intestine. Consistent with an enterocyte defect, we identified multiple important enterocyte-specific transcripts as decreased in mutant tissue (Table 1). We performed qRT-PCR to compare levels of these transcripts between control and mutant epithelium and confirmed these as significantly decreased in mutants (Figure 2A). Moreover, we observed decreased alkaline phosphatase positive (AP+) cells in GATA4-GATA6 mutants compared with controls, and any AP+ cells present in mutant tissue stained less intensely compared with control tissue (Figure 2B). Finally, using gene array data, we further identified an increase in abundance of distal intestinal marker transcripts in mutants compared with controls (Table 1). We assayed the expression of these targets by qRT-PCR (Figure 2C) and observed significant increases in these transcripts in mutant epithelium. Together, these data suggest that mutant

epithelium contained fewer enterocytes and that any remaining enterocytes expressed genes characteristic of more distal enterocytes and colonocytes rather than proximal enterocytes.

Gene array data further indicated changes in the other intestinal epithelial cell populations present at E18.5 including enteroendocrine cells, goblet cells and proliferative cells. We found expression of transcripts encoding several markers of mature enteroendocrine cells to be lower in mutant epithelium compared with controls including *ChgA*, *Cck*, and *Gip* (Table 1). *ChgA* is a pan-enteroendocrine cell product whereas *Cck* and *Gip* mark specific subpopulations of enteroendocrine cells located primarily in the proximal intestine (Schonhoff et al., 2004). qRT-PCR confirmed these gene products as less abundant in mutants compared with controls (Figure 2D). We used qRT-PCR to examine expression of *Peptide YY (Pyy)*, which marks a population of enteroendocrine cells primarily localize to the distal intestine (Schonhoff et al., 2004). We found *Pyy* to be decreased in mutants compared with controls (Figure 2D). Despite decreased expression of differentiated enteroendocrine cell markers, *Ngn3*, the transcription factor required for enteroendocrine cell specification, was unchanged (Figure 2D). Although qRT-PCR showed a significant decrease in *ChgA*, mutant intestine maintained CHGA+ cells (Figure 2E). These cells stained with an intensity equivalent to those in control tissue suggesting that *ChgA* and other enteroendocrine-specific transcript levels were lower because there were fewer fully differentiated enteroendocrine cells in mutant tissue compared with controls.

Several transcripts involved in proliferation were identified by IPA as increased in GATA4-GATA6 mutants compared with controls (Table 1), and qRT-PCR confirmed these as significantly increased in mutants (Figure 2F). Immunohistochemical staining for CD44 and SOX9, which mark the proliferative intervillus region of the small intestine (Spence et al., 2011), suggested that mutant epithelium contained increased proliferative cells compared with controls (Figure 2G). Therefore, to quantitate proliferation, we measured EdU incorporation after a 3 hour pulse. We stained tissue for EdU incorporation (proliferative cells), laminin (a mesenchyme marker used to discriminate between proliferating cells within the epithelium and mesenchyme) and DAPI (Figure 2H) and determined the proportion of proliferating cells in the small intestinal epithelium of controls and mutants. We observed 16% of cells in control epithelium as proliferating whereas 28% of cells in mutant epithelium were detected as proliferating (Figure 2I).

Finally, gene array data showed that transcripts encoding goblet cell markers were increased in mutants compared with controls (Table 1); qRT-PCR confirmed increased abundance of these transcripts in mutant tissue (Figure 3A). Examination of tissue stained with Alcian Blue (AB) or an antibody recognizing MUCIN 2 confirmed an increase in goblet cells in mutant tissue compared with control tissue (Figure 3B). To compare the number of goblet cells between control and mutant tissue, we counted AB+ cells and found a significant increase in mutants compared with controls. Control tissue contained an average of 59 AB+ cells per section whereas mutant tissue contained an average of 253 AB+ cells per section (Figure 3C). As described above, the total number of epithelial cells present in mutant tissue was comparable with control tissue (Figure 1G). Therefore, we concluded that there is a 4-fold increase in the number of goblet cells in GATA4-GATA6 deficient intestine.

Goblet cells are increased in intestine of Gata4Gata6 dcKO E16.5 embryos

Because the Villin promoter used to drive Cre activity becomes active in the intestinal epithelium as early as E12.5 (Madison et al., 2002), it is possible that the phenotype observed at E18.5 represents both primary and secondary effects of GATA4-GATA6 loss. Therefore, we used RT-PCR to determine the earliest stage at which both *Gata4* and *Gata6* were efficiently removed from the intestine and assessed the phenotype at that stage. Although *Gata6* mRNA was decreased or absent in mutant intestine by E15.5, we did not detect decreases in *Gata4* mRNA until E16.5 (Figure 4A). Therefore, we examined intestinal morphology, cell lineage allocation, and proliferation in intestines of *G4G6* dcKO embryos at E16.5, the first time at which we detected robust changes in both *Gata4* and *Gata6* levels.

Comparison of H&E stained tissue from control and mutant embryos between E16.5–E18.5 revealed that intestinal architecture defects developed over time concurrent with loss of *Gata4* and *Gata6* expression. At E16.5, when both *Gata4* and *Gata6* were first reduced, mutant intestine appeared indistinguishable from control intestine; by E18.5, intestinal epithelial structure was severely disrupted in mutant intestine compared with control intestine (Compare Figures 1E, 4B). At E17.5, we detected morphological changes intermediate to those at E16.5 and E18.5 (data not shown). Despite normal intestinal epithelial morphology at E16.5, we detected changes in enterocyte, goblet cell, and proliferative cell marker expression; enteroendocrine cell marker expression was similar between controls and mutants. qRT-PCR showed decreased abundance of enterocyte markers and increased abundance of goblet and proliferative cell markers at E16.5 (Figure 4C). Although enterocyte marker transcripts were decreased and proliferative marker transcripts were increased, staining for AP activity and SOX9 appeared similar between controls and mutants (Figure 4B). We further quantified proliferation using EdU incorporation as described above and found no increase in the proportion of proliferative cells within the epithelium of E16.5 *G4G6* dcKO intestine compared with controls (Figure 4D, E). Goblet cell number, however, was increased in E16.5 GATA4-GATA6 deficient intestinal epithelium compared with controls (Figure 4B, F). Counting AB+ cells showed that control tissue contained an average of 12 goblet cells per section whereas mutant tissue contained an average of 28 goblet cells per section representing a greater than two-fold increase in goblet cells in E16.5 GATA4-GATA6 deficient intestine (Figure 4F). Counting of HNF4A+ cells per section demonstrated there was no change in epithelial cell number (Figure 4F).

The Dll1 gene contains GATA binding sites that are occupied by GATA4 in the mouse small intestinal epithelium

Previous analysis of GATA4 expression in the intestinal epithelium shows it to be absent in goblet cells (Bosse et al., 2006). Although Beuling *et al.*(Beuling et al., 2011) report goblet cells as GATA6 positive, we found that MUC2+ cells lacked GATA6 nuclear staining when a GATA6-specific antibody was used (Supplemental Figures 3A,4). We attribute this discrepancy between results to the H-92 GATA6 antibody used by Beuling *et al*., which we demonstrated to detect a non-specific antigen in *Gata6* knockout small intestine (Supplemental Figure 4). Because both GATA4 and GATA6 are absent from mature goblet

cells and the observed increase in goblet cells corresponded with the onset of GATA4-GATA6 depletion, we hypothesized that GATA factors function in the progenitor cell population to inhibit goblet cell differentiation in the developing intestine. Importantly, both GATA4 and GATA6 are expressed in the proliferative progenitor population in the small intestinal epithelium (Supplemental Figure 3B and (Bosse et al., 2006)). As Notch signaling plays a critical role in determining secretory versus absorptive cell differentiation, we examined gene array data for evidence of altered Notch signaling. Specifically, as decreased Notch activity would favor secretory lineage differentiation, we focused on changes in gene expression predicted to decrease Notch signaling and found that expression of the Notch ligand *Dll1* was reduced in mutants (Supplemental Table 3). qRT-PCR confirmed decreased *Dll1* abundance in mutants (Figure 5A). Similar to *G4G6* dcKOs, goblet cells are increased in mice lacking DLL1 in the small intestine (Pellegrinet et al., 2011; Stamataki et al., 2011). Loss of GATA6 in the ileum of adult mice also results in decreased *Dll1* expression and increased goblet cell number in the mature ileum (Beuling et al., 2011). Therefore, to assess Notch signaling activity in GATA4-GATA6 mutants, we examined expression of the Notch target gene *Olfm4* in control and mutant tissue. *Olfm4* was chosen in light of recent work demonstrating that it serves as a sensitive indicator of Notch signaling in the intestine (VanDussen et al., 2012). qRT-PCR showed that *Olfm4* expression was significantly decreased in mutant tissue compared with control tissue (&minus8.7 fold, $P \leq 0.05$) (Figure 5A) suggesting that Notch signaling was indeed altered in the intestine of *G4G6* dcKOs.

Using MatInspector software, we queried a region of the *Dll1* gene including 1000 bp upstream of the *Dll1* transcriptional start site and the first exon of *Dll1* for GATA binding sites. Because GATA binding sites occur quite frequently in the genome, we limited our investigation to this 1637 bp region to capture the core promoter and potential regulatory sites located within the DNA encoding the 5′UTR. GATA factors, including GATA4 and GATA6, have been shown to bind to consensus binding sites within 5′UTRs in other genes to regulate gene expression (Wang et al., 2000; de Vooght et al., 2008; LaVoie et al., 2010). Within this sequence, MatInspector identified three GATA binding sites all residing within a region of *Dll1* exon 1 encoding the 5′UTR (Figure 5B). Although each sequence represents a GATA binding consensus sequence, only GATABS3 conforms to the rigorous GATA consensus sequence WGATAR. Moreover, this site was specifically annotated as a putative GATA4 binding site (V$GATA4.01) whereas the other two were annotated as putative GATA1 binding sites (V$GATA1.06).

To determine if GATA factors occupy these sites *in vivo* in the intestinal epithelium, we performed biotin-mediated chromatin immunoprecipitation (bioChIP) using $Gata4^{flbio/flbio}::Rosa26^{BirA/BirA}$ mice (Driegen et al., 2005; He et al., 2012). We chose this approach because of lack of GATA4 antibodies suitable for ChIP in the intestinal epithelium. We were limited to analyzing GATA4 because of lack of a suitable GATA6 ChIP antibody and lack of a biotin-modified mouse line. The $Gata4^{flbio/flbio}::Rosa26^{BirA/BirA}$ mouse line used is engineered such that endogenous GATA4 protein is biotinylated at its C-terminus. Streptavidin beads were used to precipitate biotinylated GATA4-chromatin complexes, and PCR was used to detect GATA4 occupancy at its predicted binding sites within *Dll1*. Four $Gata4^{flbio/flbio}::Rosa26^{BirA/BirA}$ and four

Rosa26$^{BirA/BirA}$ mice were used to determine GATA4 occupancy, and chromatin isolated from each animal was assayed in at least two independent bio-ChIP experiments. We found GATA4 to be enriched at its binding sites in *Dll1* in chromatin from *Gata4*$^{flbio/flbio}$*::Rosa26*$^{BirA/BirA}$ mice compared with control samples from *Rosa26*$^{BirA/BirA}$ mice (Figure 5C; data shown represent a total of nine independent PCR assays of GATA4 occupancy performed with chromatin from multiple bio-ChIP experiments). Therefore, we conclude that GATA4 occupies chromatin within the *Dll1* gene. It is important to note that although we can conclude that GATA4 occupies *Dll1*, we cannot distinguish between its occupancy of specific predicted binding site(s) because of their proximity to one another; only 226 bp separate GATABS1/2 and GATABS3. Taken together, our observations that *Dll1* and *Olfm4* were reduced in mutants and that GATA4 was bound to putative transcriptional regulatory elements of *Dll1* imply that GATAs regulate Notch activity in the small intestinal epithelium to control cytodifferentiation.

Discussion

Our data demonstrate that GATA4 and GATA6 play a redundant yet essential role in regulating cytodifferentation during small intestinal epithelial development. Enterocytes and enteroendocrine cell populations were compromised, whereas goblet cell and proliferative cell populations were enhanced. Because we observed no change in total epithelial cell number in mutants compared with controls, we conclude that alterations in abundance of specific epithelial cell types in mutants reflect changes in differentiation of progenitors to these lineages in the absence of GATA4 and GATA6.

One striking change in cytodifferentiation observed was increased goblet cell number. In fact, this phenotype was observed as early as E16.5, the first time at which both *Gata4* and *Gata6* levels were reduced. Because GATA4 and GATA6 are absent from mature goblet cells but expressed in the the intestinal epithelial progenitor cell population, we conclude that GATA factors function in progenitor cells to regulate cell fate decisions. We hypothesized that GATAs inhibit secretory cell differentiation in the developing small intestine by modulating Notch signaling through transcriptional regulation of the Notch ligand *Dll1*. Like *G4G6* dcKO mice, goblet cells are significantly increased in mice lacking DLL1 in the small intestine (Pellegrinet et al., 2011; Stamataki et al., 2011). Futher supporting our proposal that GATA4-GATA6 loss dampens Notch signaling in the developing intestine, we found expression of *Olfm4*, a sensitive readout of Notch signaling in the intestine, to be significantly reduced in mutant tissue compared with controls. Although we examined expression of other downstream Notch targets including *Hes1* and *Hes6*, we found levels of these to be unchanged between controls and mutants (data not shown). Notch signaling in the intestine, however, is complex, and its precise functions in embryonic intestine during development are less clear than its role in the mature intestinal epithelium. Moreover, even when DLL1 is completely absent from the intestinal epithelium in a conditional knockout model, *Hes1* levels decrease by only 44%, and *Hes5* levels remain constant (Stamataki et al., 2011). Increased expression of *Dll4* in DLL1 deficient intestine may partially explain this result (Stamataki et al., 2011). Similarly, our gene array analysis of GATA4-GATA6 deficient intestine identified a 2.5 fold increase in *Dll4* mRNA in

mutants compared with controls (Supplemental Table 3). *Olfm4* expression was not examined in the intestine of *Dll1* cKOs.

Further supporting our hypothesis that GATAs inhibit secretory cell differentiation by regulating Notch through DLL1 is our finding of GATA4 enrichment at predicted GATA bindings sites within the *Dll1* gene. Such data suggest that GATA4 directly regulates *Dll1* transcription in the developing intestine. Because of the proximity between the three predicted GATA binding sites and the nature of ChIP experiments, we can conclude that GATA4 occupies *Dll1*, but we cannot distinguish which site(s) among these is responsible. The facts that GATABS3 is predicted as a GATA4 binding site and that this site conforms to the WGATAR consensus sequence suggest it as the best candidate among the three for GATA4 binding. Because we limited our screen for GATA binding sites to 1000 bp upstream of the *Dll1* transcriptional start site and exon 1, it is possible that additional GATA binding sites residing outside this region also contribute to *Dll1* transcriptional regulation. Nevertheless, we have definitively shown using ChIP that GATA4 occupies chromatin within the *Dll1* locus. Although we were unable to test GATA6 occupancy at these sites because of a lack of suitable ChIP antibodies, we propose that GATA6 functions similarly. Supporting this proposal, we found small increases in goblet cells in both *Gata4* cKO (+1.6-fold) and *Gata6* cKO (+1.7-fold) embryos at E18.5 (Supplemental Figure 5). When both factors were simultaneously deleted, however, goblet cells quadrupled suggesting an additive effect between these factors in *Dll1* regulation.

Beuling *et al.* had previously proposed that GATA4 and/or GATA6 regulate secretory cell differentiation downstream of Notch signaling through repression of *Spdef*, a transcription factor regulating goblet cell maturation (Beuling et al., 2011). However, a recent study by the same group demonstrates that increased SPDEF in the absence of GATA6 does not drive changes in secretory cell lineage allocation as concurrent loss of *Spdef* with *Gata6* does not rescue the *Gata6* cKO ileal secretory cell phenotype (Aronson et al., 2014). In this manuscript, we provide an alternative mechanism to explain how GATA factors influence intestinal epithelial cell fate. The experimental evidence we present supports a novel mechanism through which GATAs affect intestinal epithelial cytodifferentiation by directly regulating Notch signaling itself through transcriptional control of the Notch ligand *Dll1* during embryonic development of the intestine.

Loss of GATA4-GATA6 in the developing intestine also caused a reduction in enterocyte number and a change in enterocyte identity evidenced by decreased AP activity in mutant tissue and abnormal expression of transcripts encoding genes characteristic of distal enterocytes and colonocytes in mutant epithelium. Changes in cellular identity agree with previous analyses of *Gata4* and *Gata6* cKO animals. *Gata4* cKO animals exhibit a dramatic conversion of jejunal enterocytes toward an ileal fate; *Gata6* cKO animals exhibit increased colonic marker expression in the ileum (Battle et al., 2008; Beuling et al., 2011). Because GATA4 and GATA6 are expressed in enterocytes and changes in enterocyte gene expression coincided with GATA4-GATA6 elimination at E16.5, we conclude that the enterocyte phenotype is a primary effect of GATA4-GATA6 deletion and that both are required for enterocyte differentiation. Moreover, we conclude that GATA4 and GATA6 are required for proper intestinal architecture. In the absence of GATA4 and GATA6, abnormal

enterocyte function causes increased lumenal fluid accumulation and gut dilation. These mechanical changes occuring in mutants likely explain alterations in villus shape and number. Taken together, abnormal villus structure, decreased enterocyte number, and deviations from the proper jejunal expression profile in remaining enterocytes likely cause malnutrition in mutant neonates leading to death.

Because enteroendocrine cells constitute only 1% of the intestinal epithelium, we did not directly quantitate their abundance but instead used qRT-PCR to examine expression of key enteroendocrine cell markers. At E18.5, we observed reduced levels of multiple mature enteroendocrine cell markers. Therefore, we conclude that loss of GATA4 and GATA6 in the developing small intestinal epithelium impairs enteroendocrine cell differentiation. Changes in expression of mature enteroendocrine cell markers also occur in single *Gata4* and *Gata6* Villin-Cre cKO mice and in mice with deletion of both *Gata4* and *Gata6* in adult intestine (Bosse et al., 2006; Battle et al., 2008; Beuling et al., 2011). Furthermore, *Ngn3* levels are decreased in ileum of adult *Gata6* cKO mice and in duodenum and jejunum of mice with deletion of both *Gata4* and *Gata6* in adult intestine leading to the conclusion that GATA factors are required for enteroendocrine lineage commitment (Bosse et al., 2006; Beuling et al., 2011). In contrast, we found no difference in *Ngn3* expression between controls and GATA4-GATA6 mutants at E18.5 implying that GATAs function differently during development affecting enteroendocrine cell differentiation rather than committment to enteroendocrine cell fate. Moreover, we found no change in enteroendocrine cell marker expression at E16.5 suggesting that changes in enteroendocrine cell populations represent a secondary consequence of GATA4-GATA6 loss. As GATA factors regulate intestinal enterocyte identity, it is possible that GATAs indirectly affect enteroendocrine cells downstream of regional identity regulation.

It is unclear why with greater proliferation in mutants compared with controls, we failed to observe an increase in epithelial cell number. We examined cell death in mutants by staining for active caspase 3 (data not shown) and found no change demonstrating that this phenomenon cannot be explained by a concomitant cell loss. In contrast to elimination of GATA4 and GATA6 in the developing intestine, elimination of GATA factors in the adult small intestine or the pancreas inhibits proliferation (Beuling et al., 2011; Carrasco et al., 2012; Xuan et al., 2012). It is possible that the enterocyte differentiation blockade in GATA4-GATA6 mutants caused proliferating progenitors to accumulate. Although we observed changes in proliferation marker expression at E16.5, arguing a primary role for GATAs in controlling proliferation, it is also possible that increased proliferation simply reflects a compensatory response to severe disruption to the enterocyte population and villus architecture. Similar changes are observed in other intestinal mutants in which enterocytes and villus structure are perturbed (Hermiston and Gordon, 1995; Bondow et al., 2012). Finally, our finding that proliferation is increaed in the context of decreased DLL1-driven Notch signaling highlights the complexity of the Notch pathway in the developing intestinal epithelium. It is likely that individual Notch ligands in combination with cognate Notch receptors regulate distinct signaling outcomes such as proliferation or cytodifferentiation.

Conclusions

Based on our data, we propose that GATA4 and GATA6 repress goblet cell differentiation through transcriptional regulation of the Notch ligand *Dll1* during epithelial cell lineage specification (Figure 6). Recently, the Clevers group demonstrated that cells expressing high levels of *Dll1* exist within the progenitor population present in epithelium of the adult small intestine, and it is this population that constitutes the secretory progenitor pool (van Es et al., 2012). Such cells provide the Notch ligand DLL1 to adjacent neighboring progenitors to drive Notch signaling within these neighbors thereby promoting differentiation of these cells to the enterocyte fate. We propose that GATA4 and GATA6 function to enhance *Dll1* expression within the secretory progenitor pool. When these factors are lost, *Dll1* expression is reduced; concomitantly, the total amount of Notch ligand available to secretory progenitor cell neighbors is reduced. This reduction in Notch signaling thereby skews the balance between enterocytes and secretory cells resulting in a loss of enterocytes and a gain in goblet cells. Finally, as GATA4 and GATA6 are absent in mature goblet cells, we propose that signals within differentiating goblet cells subsequently repress GATA4 and GATA6 expression during cell maturation. The finding that DLL1 levels are lower in differentiated goblet cells supports the hypothesis that GATA levels drop as progenitor cells differentiate and mature along the goblet cell lineage (Stamataki et al., 2011).

In conclusion, this study expands our understanding of the signaling pathways and transcription factors required for absorptive and secretory cell differentiation during intestinal development. We demonstrate that both GATA4 and GATA6 play an essential role in defining epithelial architecture and in controlling cytodifferentiation in the small intestine. Moreover, our data highlight a novel role for GATA factors in fine tuning Notch signaling in the intestine through transcriptional control of the Notch ligand *Dll1* to repress goblet cell differentiation thereby promoting enterocyte differentiation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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- GATA4-GATA6 deletion in the developing intestine caused neonatal lethality.
- Mutant epithelium contained fewer enterocytes and increased goblet cells.
- GATA4 occupies chromatin within *Dll1*, a gene encoding a Notch ligand.
- Our data highlight a role for GATA4/6 in modulating Notch signaling in the intestine.
- We propose that GATA4/6 repress secretory cell differentiation by fine tuning Notch.

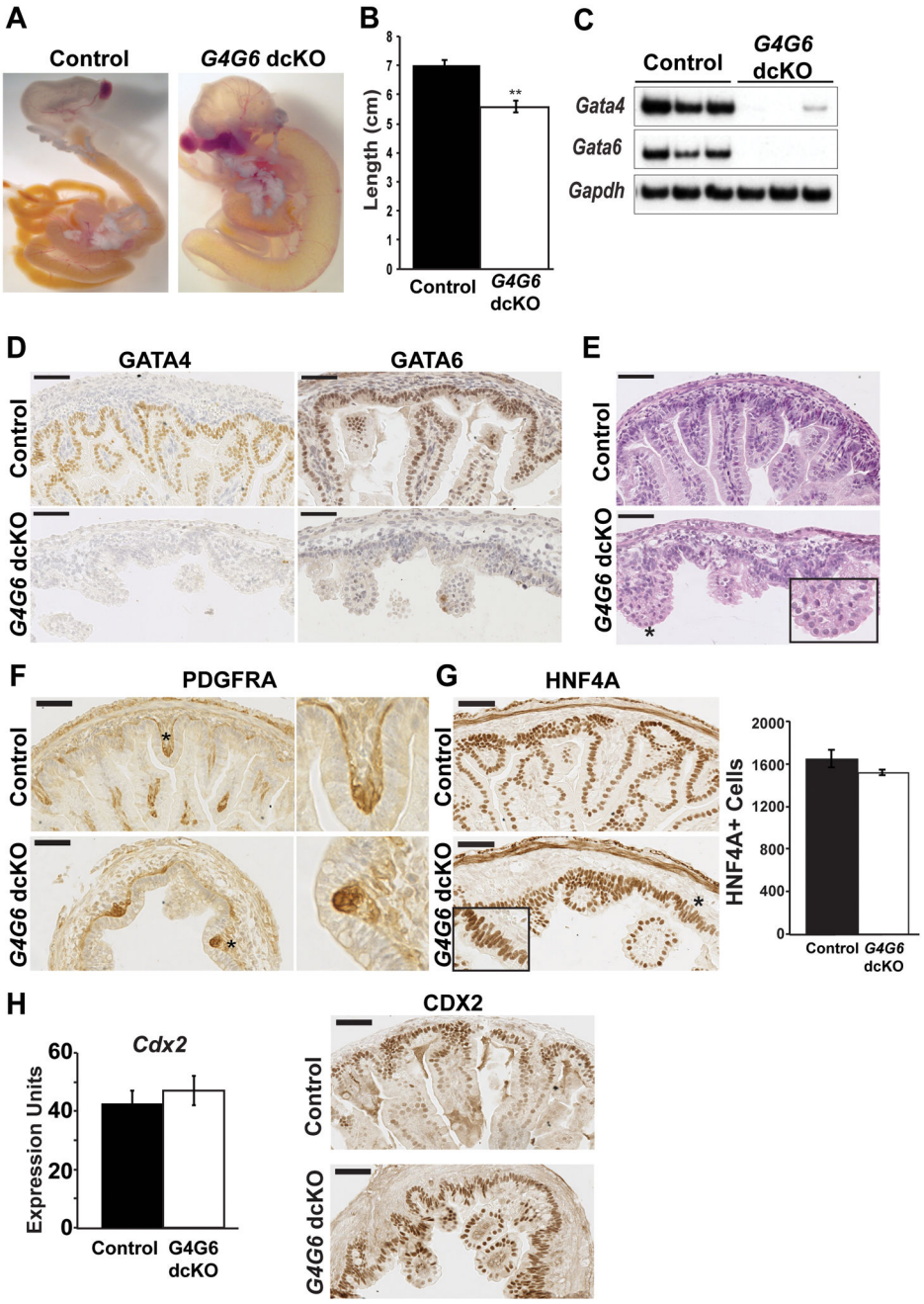

Figure 1.

Loss of GATA4 and GATA6 disrupts intestinal epithelial architecture at E18.5. (A) Intestine from *G4G6* dcKO embryos appeared dilated and translucent compared with controls. (B) Average small intestine length was decreased in *G4G6* dcKO embryos (n=29) compared with controls (n=29). (C) RT-PCR demonstrated efficient *Gata4-Gata6* knockout in intestinal epithelial cells of *G4G6* dcKO embryos. *Gapdh* was used as loading control. (D) GATA4 and GATA6 immunohistochemistry showed an absence of nuclear staining (brown) in jejunum of mutants compared with controls. (E) H&E staining of jejunal tissue demonstrated villus loss and altered cell shape in the absence of GATA4-GATA6. Inset

shows higher magnification of starred region. (F) PDGFRA immunohistochemistry showed comparable levels and localization between control and mutant intestine. Right panels show higher magnification of starred regions. (G) HNF4A staining showed that mutant jejunal tissue contained densely packed nuclei within the intervillus regions. Inset shows higher magnification of starred region. Epithelial cell number was not changed between control (average 1646 HNF4A+ cells/section) and *G4G6* dcKO (average 1520 HNF4A+ cells/section) embryos (n= 3 sections from 3 control and 3 mutant intestines). (H) Intestinal fate was preserved in intestine of *G4G6* dcKOs. Expression of CDX2, an early marker of intestinal fate, was assayed by qRT-PCR (left) and immunohistochemistry (right). Both *Cdx2* mRNA and CDX2 protein were comparable between controls and mutants. All scale bars = 50μm. Error bars show standard error of the mean (SEM). *P*-value was determined by two-sample Student *t* test: $^{**}P \leq 0.01$.

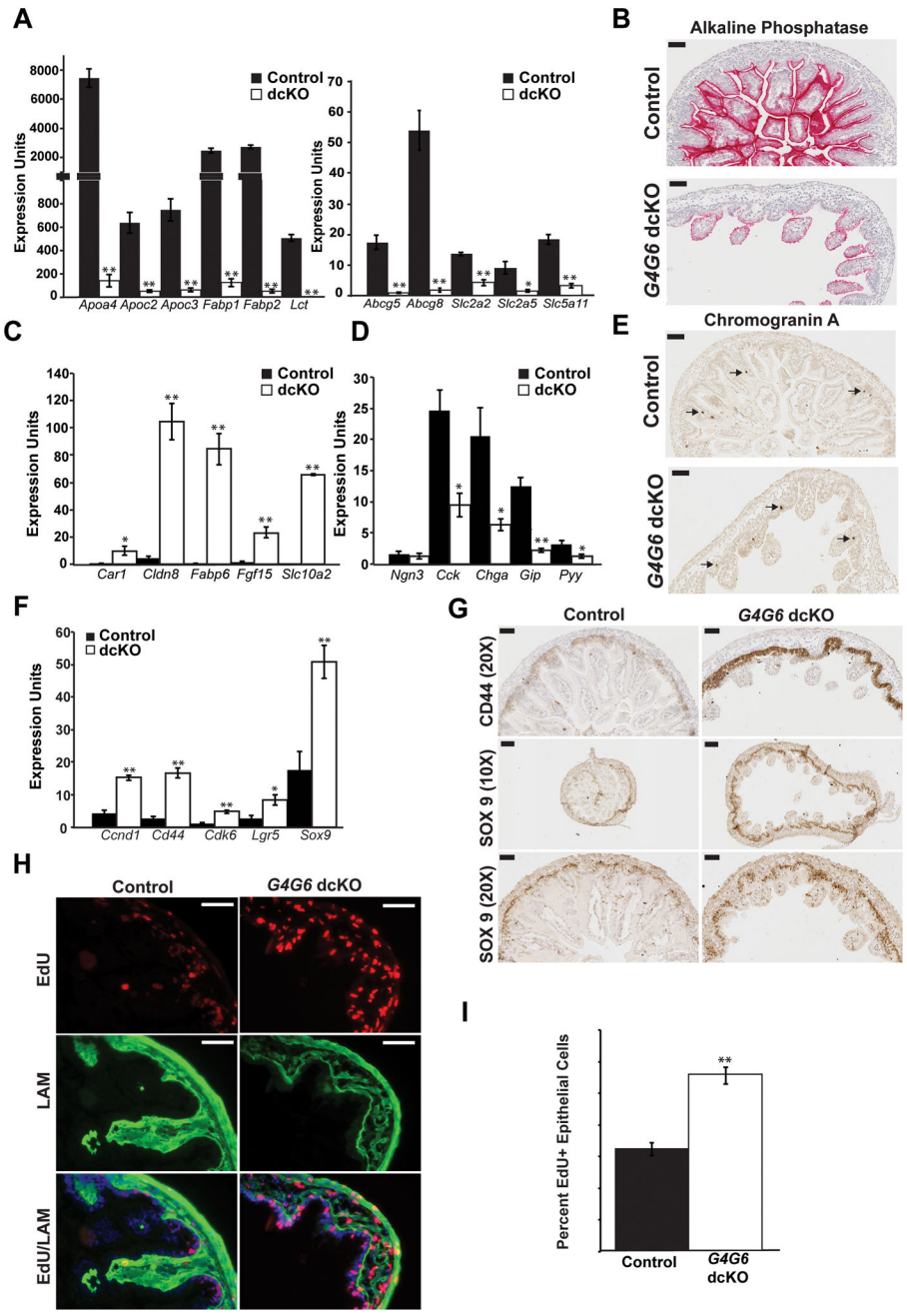

Figure 2.

Enterocyte and enteroendocrine cells are negatively affected by loss of GATA4 and GATA6 at E18.5. (A) qRT-PCR analysis of intestinal epithelial cells from control and *G4G6* dcKO embryos confirmed that the abundance of transcripts encoding jejunal enterocyte-specific markers was significantly decreased in mutants compared with controls. (B) Alkaline phosphatase activity was decreased in mutant jejunum compared with control tissue. Scale bars = 50μm (C) qRT-PCR analysis of intestinal epithelial cells from control and *G4G6* dcKO embryos confirmed that the abundance of transcripts encoding distal enterocyte-specific markers was significantly increased in mutants compared with controls. (D) qRT-

PCR analysis of intestinal epithelial cells from control and *G4G6* dcKO embryos confirmed changes in enteroendocrine marker expression in mutants compared with controls. *Ngn3* was unchanged. (E) Chromogranin A+ cells (arrows) were detected in jejunal tissue from control and *G4G6* dCKO embryos showing the presence of enteroendocrine cells in both tissues. Scale bars = 50μm (F) qRT-PCR analysis of intestinal epithelial cells from control and *G4G6* dcKO embryos confirmed that the abundance of transcripts encoding proliferative cell markers was significantly increased in mutants compared with controls. (G) Immunohistochemistry for proliferation markers CD44 (brown membrane stain) and SOX9 (brown nuclear stain) showed increased levels of both in jejunum of mutants. Scale bars, 10X micrographs = 100μm; 20X micrographs = 50μm. (H) EdU incorporation showed an increase in proliferating cells within the jejunal epithelium of mutants. Co-staining for EdU (red) and laminin (LAM, green) differentiated between proliferating epithelial cells (EdU+, LAM−) and proliferating mesenchymal cells (EdU+, LAM+). DAPI (blue) identified nuclei. Scale bars = 50μm (I) The proportion of proliferating cells within the jejunal epithelium was determined by dividing the number of EdU+DAPI+LAM- by the number of DAPI+LAM- cells (n= 10 fields per embryo, 3 control and 3 mutant embryos). Error bars show SEM. *P*-value was determined by two-sample Student *t* test: $^{**}P<0.01$. For all qRT-PCR, cells from 3 control and 3 mutant intestines were assayed at least 3 times. *Gapdh* was used for normalization. Error bars show SEM. *P*-value was determined by two-sample Student *t* test: $^{*}P\leq0.05$, $^{**}P\leq0.01$

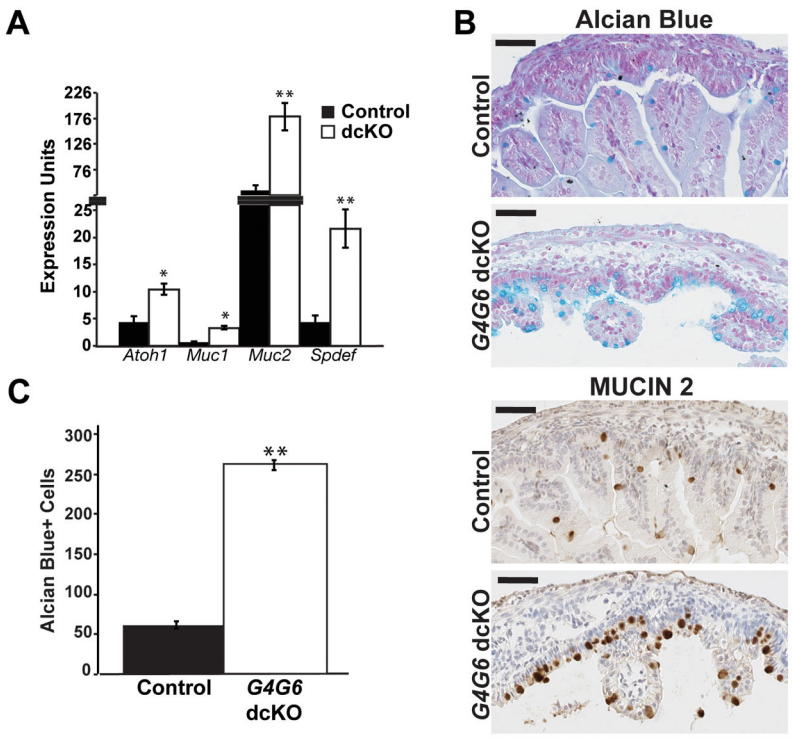

Figure 3.

Goblet cells are increased in GATA4-GATA6 deficient intestine at E18.5. (A) qRT-PCR analysis of intestinal epithelial cells from control (n=3) and *G4G6* dcKO (n=3) embryos confirmed that the abundance of transcripts encoding goblet cell markers was significantly increased in mutants compared with controls. Assays were performed at least 3 times. *Gapdh* was used for normalization. Error bars show SEM. *P*-value was determined by two-sample Student *t* test: **P*≤0.05, ***P*≤0.01. (B) Alcian Blue (AB) staining and MUCIN 2 immunohistochemistry (brown) identified goblet cells in control and *G4G6* dcKO jejunum. Scale bars = 50μm. (C) Quantitation of AB+ cells demonstrated a significant increase in goblet cells in GATA4-GATA6 deficient intestinal epithelium compared with controls (n= ≥3 sections from 3 control and 3 mutant intestines). Error bars show SEM. *P*-value was determined by two-sample Student *t* test: ***P*<0.01.

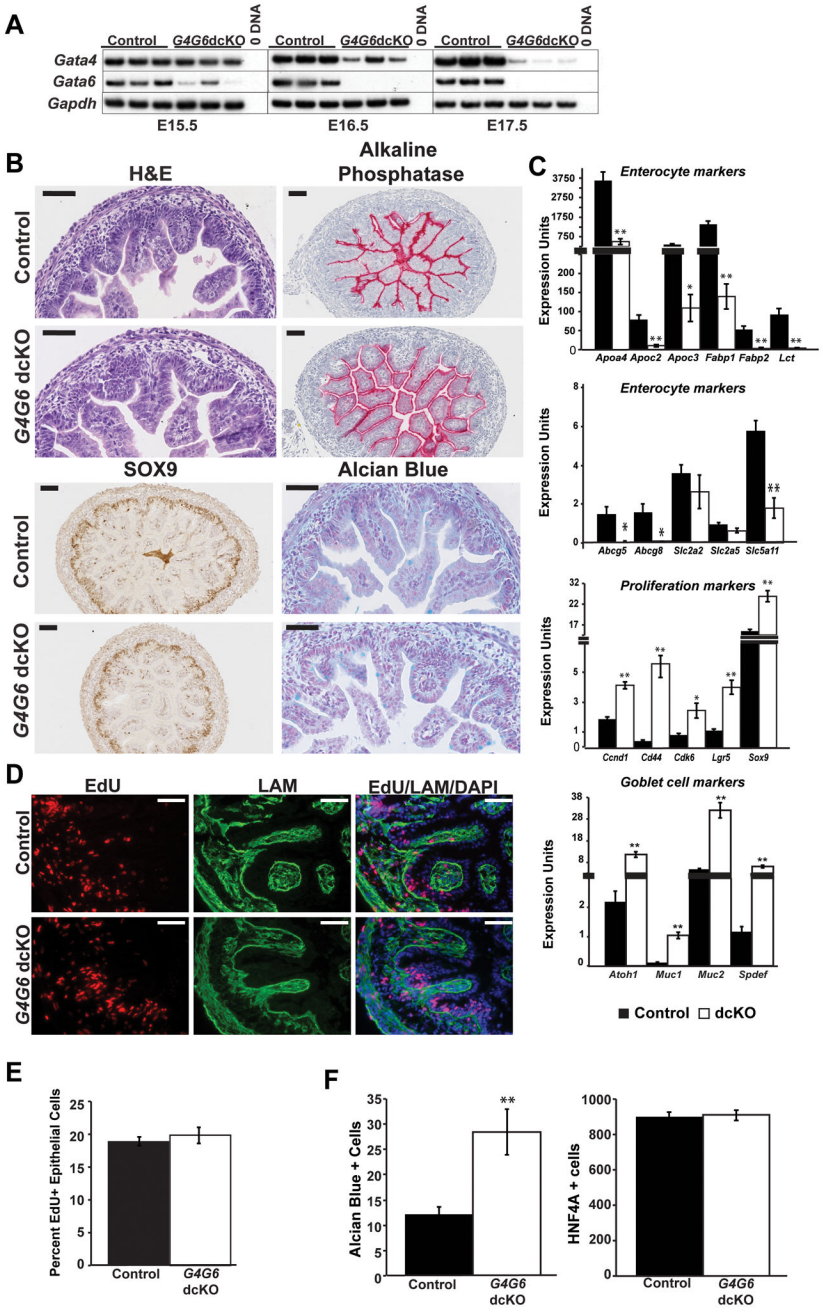

Figure 4.

Epithelial cell populations are altered in GATA4-GATA6 deficient intestine at E16.5. (A) *Gata4* and *Gata6* knockout in small intestine between E15.5–E17.5 was assessed using RT-PCR. *Gapdh* was used as loading control. At E15.5, although *Gata6* levels were decreased in mutant intestine, *Gata4* levels remained high. At E16.5, *Gata4* transcript was decreased although levels varied between embryos. At E17.5, *Gata4* was virtually absent in mutants. *Gapdh* was used as loading control. (B) H&E, alkaline phosphatase activity, and SOX9 immunohistochemistry were comparable between jejunum from control and *G4G6* dcKO embryos at E16.5. Alcian Blue (AB) staining demonstrated increased goblet cells at E16.5.

(C) qRT-PCR analysis of intestinal epithelial cells from control (n=3) and *G4G6* dcKO (n=3) E16.5 embryos demonstrated that abundance of enterocyte markers was decreased and that abundance of proliferation markers and goblet cell markers was increased in mutants compared with controls. Assays were performed at least 3 times. *Gapdh* was used for normalization. Error bars show SEM. *P*-value was determined by two-sample Student *t* test: **P*≤*0*.05, ***P*≤*0*.01. (D, E) The number of proliferative cells present in control and mutant jejunal epithelium, determined by EdU incorporation as described in Figure 4C–D, was unchanged between controls and *G4G6* dcKO intestine at E16.5 (n= ≥5 fields per embryo, 4 control and 4 mutant embryos). (F) Quantitation of AB+ cells demonstrated a significant increase in goblet cells in GATA4-GATA6 deficient jejunal epithelium compared with controls (n= ≥3 sections from 3 control and 3 mutant intestines). Epithelial cell number, determined by HNF4A+ nuclei, was unchanged (Average 902 HNF4A+ cells/section controls; average 906 HNF4A+ cells/section mutants). All scale bars = 50μm. Error bars show SEM. *P*-value was determined by two-sample Student *t* test: **P*≤*0*.05, ***P*≤*0*.01.

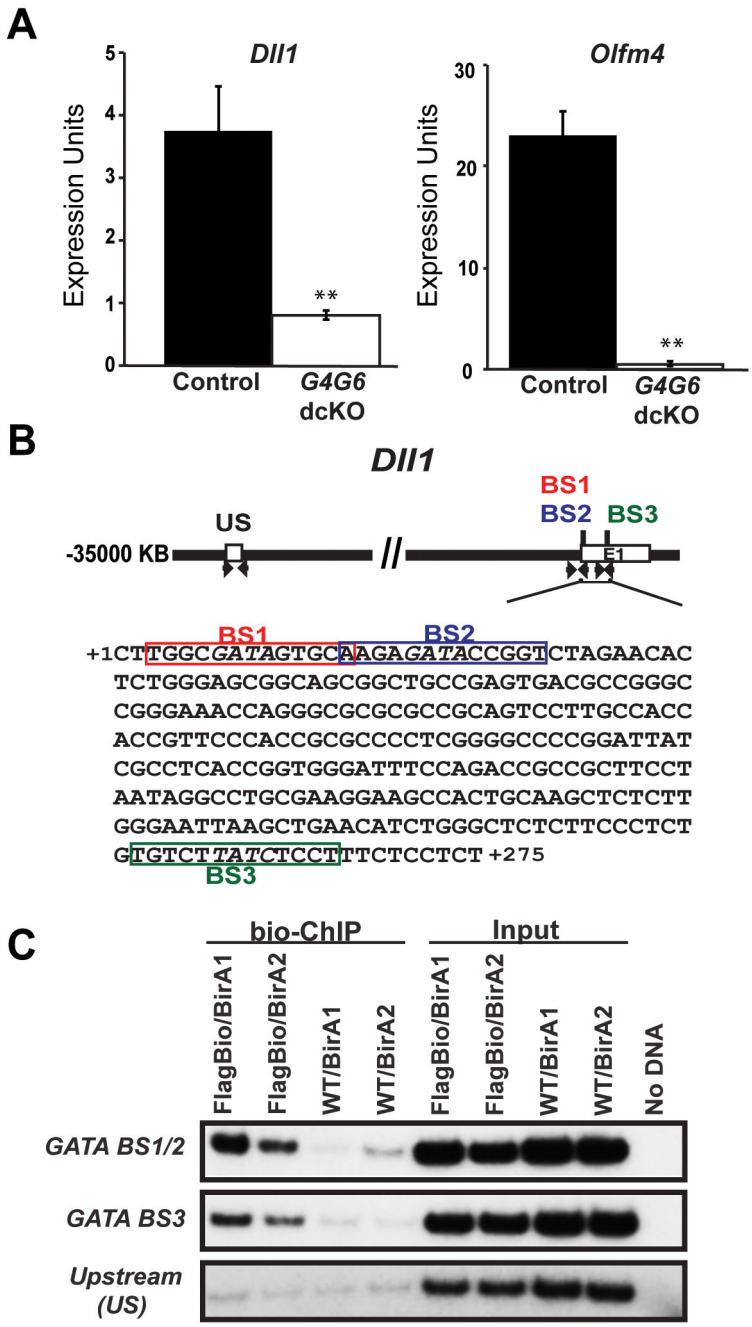

Figure 5.

GATA4 occupies binding sites in the *Dll1* gene. (A) qRT-PCR analysis of intestinal epithelial cells from control and *G4G6* dcKO E18.5 embryos demonstrated decreased levels of *Dll1* and *Olfm4* transcripts in mutants compared with controls. Cells from 3 control and 3 mutant intestines were assayed at least 3 times. *Gapdh* was used for normalization. Error bars show SEM. *P*-value was determined by two-sample Student *t* test: **P≤0.01. (B) Illustration of *Dll1* gene depicting three GATA binding sites (GATABS1/2, GATABS3) predicted by MatInspector software. All three sites reside within a region of exon 1 that

encodes the *Dll1* 5′UTR. The core GATA sequence is indicated by italics. Arrows denote bio-ChIP primers. E1, exon 1; US, upstream primer pair spanning a region without a predicted GATA binding site (−31,199 to −31,357 bp; bio-ChIP negative control). (C) PCR demonstrated GATA4 enrichment at predicted binding sites GATABS1/2 and GATABS3 in chromatin from two GATA4FlagBio/BirA animals compared with chromatin from two control GATA4 wild-type/BirA animals. No enrichment was evident using US primers. Input shows that equivalent chromatin amounts were used in each bio-ChIP assay. Four GATA4FlagBio/BirA and four BirA mice were used to generate bio-ChIP data. Chromatin isolated from each animal was assayed in least two independent bio-ChIP experiments. PCR data shown in this panel are representative of nine PCR assays of GATA4 occupancy at these sites.

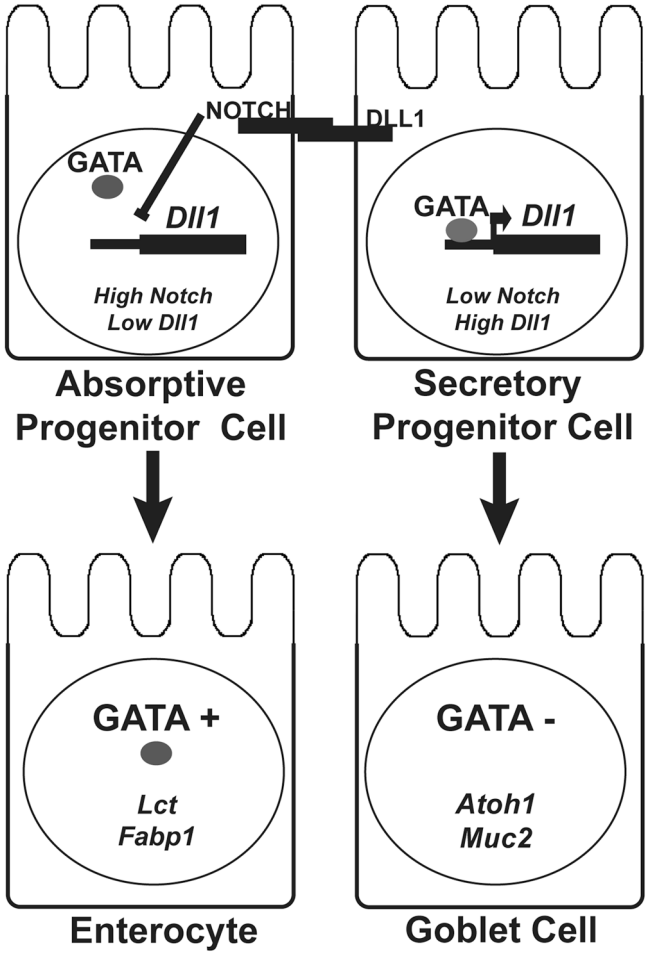

Figure 6.

Model for GATA regulation of Notch signaling in the intestine. During the process of epithelial cytodifferentiation within the intestine, a subset of progenitor cells stochastically loses Notch receptor expression and upregulates *Dll1* expression thereby defining these cells as the secretory progenitors(van Es et al., 2012). We propose that within the secretory progenitor population, GATA factors function to increase *Dll1* expression thereby contributing to the overall high level of *Dll1* characteristic of these cells. Interaction between DLL1 on secretory progenitors and Notch receptors on adjacent progenitors drives Notch signaling within neighboring cells, and such Notch-receiving progenitor cells generate enterocytes rather than secretory cells. Although GATA factors are present within the progenitor population as a whole, within Notch-receiving absorptive progenitors, activation of the Notch pathway results in repression of *Dll1* expression (Sprinzak et al., 2010). Absorptive progenitors continue to express GATA factors and differentiate into mature enterocytes expressing markers including *Lct* and *Fabp1*. As secretory progenitors differentiate into mature goblet cells, expressing markers including *Atoh1* and *Muc2*, GATA expression is lost. In the case of intestine from *G4G6* dcKO embryos, we propose that loss of GATA factors dampens overall DLL1 levels in secretory progenitors. Therefore, there is less Notch ligand available to neighboring cells, and the balance between the absorptive and

secretory cell lineages is altered to favor secretory cell fate. Moreover, loss of GATA factors in remaining absorptive progenitors results in abnormal enterocyte differentiation.

Table 1

Genes encoding transcripts related to epithelial cytodifferentiation and proliferation identified by Affymetrix gene array analysis with increased or decreased expression (≥2.0-fold, P≤0.05) in E18.5 GATA4-GATA6 deficient intestinal epithelium compared with control epithelium.

Gene	Fold Change[1]
Enterocyte Markers	
ATP-binding cassette family member 5 (Abcg5)	−8.1
ATP-binding cassette family member 8 (Abcg8)	−9.0
Apolipoprotein A4 (Apoa4)	−6.0
Apolipoprotein C2 (Apoc2)	−3.8
Apolipoprotein C3 (Apoc3)	−2.0
Fatty acid binding protein 1 (Fabp1)	−3.9
Fatty acid binding protein 2 (Fabp2)	−6.6
Lactase (Lct)	−23.4
Solute carrier family 2 member 2 (Slc2a2)	−2.5
Solute carrier family 2 member 5 (Slc2a5)	−3.10
Solute carrier family 5 member 11 (Slc5a11)	−3.3
Distal Intestine Markers	
Carbonic anhydrase 1 (Car1)	10.9
Claudin 8 (Cldn8)	6.2
Fatty acid binding protein 6, ileal (Fabp6)	32.7
Fibroblast growth factor 15 (Fgf15)	8.40
Solute carrier family 10, member 2 (Slc10a2)	17.3
Enteroendocrine Cell Markers	
Cholecystokinin (Cck)	−2.3
Chromogranin A (ChgA)	−2.1
Gastric inhibitory peptide (Gip)	−3.8
Goblet Cell Markers	
Atonal homolog 1 (Atoh1)	2.3
SAM pointed domain containing ets transcription factor (Spdef)	2.6
Mucin 1 (Muc1)	2.6
Mucin 2 (Muc2)	2.4
Proliferative Cell Markers	
Cyclin D1 (Ccnd1)	2.4
CD44 antigen (Cd44)	4.1
Cyclin-dependent kinase 6 (Cdk6)	3.1
Leucine rich repeat containing G protein coupled receptor 5 (Lgr5)	2.6
SRY-box containing gene 9 (Sox9)	2.2

[1] P≤0.05