GATA Factors Regulate Proliferation, Differentiation, and Gene Expression in Small Intestine of Mature Mice

EVA BEULING,* NANA YAA A. BAFFOUR–AWUAH,* KELLY A. STAPLETON,* BOAZ E. ARONSON,* TAEKO K. NOAH,‡ NOAH F. SHROYER,‡ STEPHEN A. DUNCAN,§ JAMES C. FLEET,‖ and STEPHEN D. KRASINSKI*,¶

**Division of Gastroenterology and Nutrition, Department of Medicine, Children's Hospital Boston, Boston, Massachusetts; ‡Division of Gastroenterology, Hepatology and Nutrition, Cincinnati Children's Hospital, and University of Cincinnati College of Medicine, Cincinnati, Ohio; §Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin; ‖Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana; and ¶Gerald J. and Dorothy R. Friedman School of Nutrition Science and Policy, Tufts University, Boston, Massachusetts*

BACKGROUND & AIMS: GATA transcription factors regulate proliferation, differentiation, and gene expression in multiple organs. GATA4 is expressed in the proximal 85% of the small intestine and regulates the jejunal-ileal gradient in absorptive enterocyte gene expression. GATA6 is co-expressed with GATA4 but also is expressed in the ileum; its function in the mature small intestine is unknown. **METHODS:** We investigated the function of GATA6 in small intestine using adult mice with conditional, inducible deletion of *Gata6*, or *Gata6* and *Gata4*, specifically in the intestine. **RESULTS:** In ileum, deletion of *Gata6* caused a decrease in crypt cell proliferation and numbers of enteroendocrine and Paneth cells, an increase in numbers of goblet-like cells in crypts, and altered expression of genes specific to absorptive enterocytes. In contrast to ileum, deletion of *Gata6* caused an increase in numbers of Paneth cells in jejunum and ileum. Deletion of *Gata6* and *Gata4* resulted in a jejunal and duodenal phenotype that was nearly identical to that in the ileum after deletion of *Gata6* alone, revealing common functions for GATA6 and GATA4. **CONCLUSIONS: GATA transcription factors are required for crypt cell proliferation, secretory cell differentiation, and absorptive enterocyte gene expression in the small intestinal epithelium.**

Keywords: GATA4; GATA6; Crypt Cell Proliferation; Intestinal Differentiation.

The mature mammalian small intestine is a highly regenerative organ in which the orderly differentiation of cells along the crypt–villus axis and the precise distribution of specialized cell types and expression of proteins are essential for intestinal function. Stem cells located at or near the base of crypts produce transit-amplifying cells that undergo a series of cell fate decisions, ultimately giving rise to 4 main cell types. Absorptive enterocytes, the most numerous villus cell type, express digestive enzymes and transporters in a tightly regulated spatial pattern designed for optimal digestion and absorption of nutrients. Mucus-secreting goblet cells and defensin-secreting Paneth cells are necessary for maintaining a dynamic mucosal defensive barrier, and enteroendocrine cell subpopulations display a functional diversity characterized by the regional segregation of hormone secretions that activate or repress gastrointestinal processes. Absorptive enterocytes, goblet cells, and enteroendocrine cells migrate up to populate the villus epithelium and turn over in 3–4 days, whereas Paneth cells migrate to the base of crypts and turn over at a slower rate of 3–6 weeks.[1]

Wnt and Notch signaling play essential roles in the regeneration of the intestinal epithelium. Disruption of Wnt signaling results in a complete loss of proliferation and a decrease in secretory cell differentiation,[2–6] whereas overactivation of Wnt signaling leads to hyperproliferation, enlarged crypts, stunted villi, and an increase in secretory cells.[7] Disruption of Notch signaling results in a decrease in crypt cell proliferation and an excessive number of secretory cells,[8–11] whereas overactivation of Notch signaling results in an increase in cell proliferation and a reduction of all secretory cell types.[12] Notch signaling regulates the balance of absorptive vs secretory cells by activating hairy and enhancer of split 1 (HES1),[13] a basic helix-loop-helix transcription factor that selects the absorptive enterocyte lineage. Progenitor cells that escape Notch signaling and activation of *Hes1* gene transcription express atonal homolog 1 (ATOH1), a basic helix-loop-helix transcription factor that selects the secretory cells (ie, enteroendocrine, goblet, and Paneth cells).[14] ATOH1-positive secretory progenitors then undergo a series of decisions ultimately resulting in a tightly regulated distribution and localization of mature secretory cells.

Abbreviations used in this paper: Apoa1, apolipoprotein A-I; ATOH1, atonal homolog 1; BrdU, bromodeoxyuridine; CAR, carbonic anhydrase; CHGA, chromogranin A; DLL1, delta-like 1; EM, electron microscopy; EPHB, eph receptor B; GFI1, growth factor independent 1; HES1, hairy and enhancer of split 1; LYZ, lysozyme; MUC2, mucin 2; NGN3, neurogenin 3; qRT-PCR, quantitative reverse-transcriptase polymerase chain reaction; SOX9, SRY-box containing gene 9; SPDEF, SAM pointed domain containing ets transcription factor; TFF3, trefoil factor 3.

0016-5085/$36.00
doi:10.1053/j.gastro.2011.01.033

GATA proteins are conserved transcription factors that regulate proliferation, differentiation, and gene expression in multiple organs.[15] GATA4 is expressed in the crypt and villus epithelium in the proximal 85% of adult small intestine but is absent from distal ileum,[16–19] whereas GATA6 is expressed in the crypt and villus epithelium throughout the small intestine, including distal ileum.[16–18,20] Deletion or mutation of GATA4 results in a decrease in the expression of absorptive enterocyte genes normally found in jejunum, and an increase in the expression of ileal genes showing that GATA4 mediates jejunal–ileal identities in absorptive enterocyte gene expression and function.[16,21,22] Expression of a dominant-negative GATA4 mutant produces not only the jejunal–ileal changes in gene expression, but also alterations in enteroendocrine and goblet cells,[16] suggesting a role for GATA factors in secretory cell differentiation. The function of GATA6 in the adult small intestine is unknown. The hypothesis to be tested in this study is that GATA factors are necessary for secretory cell differentiation, and possibly other functions, in the small intestine.

Materials and Methods

Mice

Previously established and confirmed *Gata6*$^{loxP/loxP}$, *Gata4*$^{flap/flap}$, and transgenic Villin*Cre*ER^{T2} mice[16,20,22,23] were used in this study to produce conditional, inducible deletion of *Gata6* or both *Gata6* and *Gata4* in the intestinal epithelium. *Gata6*$^{loxP/loxP}$, Villin*Cre*ER^{T2}-positive (*G6del*), *Gata6*$^{loxP/loxP}$, *Gata4*$^{flap/flap}$, Villin*Cre*ER^{T2}-positive (*G6G4del*), and *Gata6*$^{loxP/loxP}$, Villin*Cre*ER^{T2}-negative or *Gata6*$^{Wt/Wt}$, Villin*Cre*ER^{T2}-positive (*control*) mice, 6–8 weeks of age, were treated with tamoxifen as described.[16,22] Tissue was collected 28 days after treatment, unless otherwise indicated. In a subset of male mice, food intake was determined by measuring consumption during the last 3 days before terminal tissue collection. After the 3-day food intake analysis, body weight was measured, the mice were killed, and blood and tissues were collected. From serum, cholesterol and triglyceride levels were determined. Approval was obtained from the Institutional Animal Care and Use Committee.

Tissue Isolation

Mice were dissected as previously described.[16] Samples of ileum were taken from distal small intestine adjacent to the ileocecal valve, samples of jejunum from the geometric center of the small intestine, and samples of duodenum from the first centimeter adjacent to the pylorus. In selected mice, bromodeoxyuridine (BrdU) (0.1 mL of 10 mg/mL) was injected 2 hours before dissection.

Immunohistochemistry and Electron Microscopy

Immunostaining of intestinal segments was conducted as previously described[16] (see Supplementary Materials and Methods section for a list of antibodies). For electron microscopy (EM), intestinal segments were fixed in 1.25% glutaraldehyde, 4% formaldehyde, 0.1 mol/L cacodylic buffer, pH 7.4, at 4°C overnight. EM was conducted in the Harvard Digestive Disease Center imaging core at Beth Israel Deaconess Medical Center.

Villus and Crypt Measurements and Cell Counting

Villus length and crypt depth were measured using ImageJ software (available at: http://rsb.info.nih.gov/ij/). The total number of villus and crypt cells was determined by counting the visible nuclei in the epithelial layer. The total number of alcian blue–positive cells on villi was determined as a percentage of total villus epithelial cells. The total number of alcian blue–, Ki67-, BrdU-, or lysozyme (LYZ)-positive cells in crypts was determined as the total number per crypt, and the average number of chromogranin A (CHGA)–positive cells was expressed as a fraction of total epithelial cells (villi and crypts) from a minimum of 5000 epithelial cells counted. For all determinations (blinded and conducted on a minimum of 5 animals per group, unless otherwise indicated), a minimum of 6 villi or 6 crypts per slide were analyzed.

RNA Isolation and Gene Expression Analysis

RNA was isolated from 0.5- to 1.0-cm intestinal segments, and gene expression was determined by quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) as previously described[16,22,24,25] using validated primer pairs (Supplementary Figure 1). Ileal messenger RNA (mRNA) also was analyzed by whole-genome gene expression analysis using the Affymetrix Mouse Gene 1.0 ST array by the molecular genetics core facility at Children's Hospital Boston. A minimum of 5 mice in each group were analyzed by qRT-PCR unless indicated otherwise; 3 mice in each group were analyzed by microarray.

Statistical Analyses

Data are expressed as mean ± standard deviation. Statistically significant differences were determined by the 2-tailed Student *t* test. Differences were considered statistically significant at a *P* value of less than .05.

Results

Intestinal Gata6 Deletion Results in a Reduction in Villus Length, Villus Epithelial Cell Number, and Crypt Proliferation in Ileum

GATA6 was expressed in all differentiated and proliferating cells in the mature mouse small intestinal epithelium, with the highest staining intensity in the proliferative crypt compartment (Supplementary Figure 2). To determine the function of GATA6 in this tissue, a